U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-284 392

Methods for the Determination of Chemical Contaminants in Drinking Water - Participants Handbook

Environmental Protection Agency, Cincinnati, Ohio

Nov 77

EPA 430/1-78-008

PB 284 392

Methods For The Determination Of Chemical Contaminants In Drinking Water

PARTICIPANTS HANDBOOK

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER PROGRAM OPERATIONS

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA 430/1-78-008

2.

3. RECIPIENT'S ACCESSION NO.
PB284392

4. TITLE AND SUBTITLE
Methods for the Determination of Chemical Contaminants in Drinking Water - Participants Handbook

5. REPORT DATE
June 1978

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
John D. Pfaff

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Protection Agency
Municipal Operations & Training Division
National Training & Operational Technology Center
Cincinnati, Ohio 45268

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Same as #9 above

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
700/02

15. SUPPLEMENTARY NOTES
Alternate Methods not included.

16. ABSTRACT

This laboratory manual is designed to contain analytical procedures for all parameters listed in the National Interim Primary Drinking Water Regulations (NIPDWR). Some procedures may be carried out by operators or laboratory technicians with little or not experince (chlorine, turbidity, fluoride, nitrate) while other parameters require understanding and experience in using sophisticated analytical equipment (atomic absorption, gas chromatography). Parameters included are procedures for silver, cadmium, chromium, lead, mercury, arsenic, selenium, nitrate, fluoride, barium, chlorine, turbidity, pesticides, herebicides. It is written in a step-by-step format.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Analysis, Chemical Analysis, Chlorine, Turbidity, Metals, Silver, Cadmium Chromium Fluoride, Barium, Pesticides, Herbicides, Potable water.		07B, 07C, 14B

18. DISTRIBUTION STATEMENT
Release to the Public

19. SECURITY CLASS *(This Report)*
Unclassified

20. SECURITY CLASS *(This page)*
Unclassified

21. NO. OF PAGES
429

22. PRICE
PC A19
MF A01

EPA Form 2220-1 (9-73)

November 1977

PARTICIPANTS HANDBOOK

for

METHODS FOR THE DETERMINATION OF CHEMICAL CONTAMINANTS IN DRINKING WATER

This Participants Handbook was developed by the Environmental Protection Agency, National Training and Operational Technology Center with the Technical Support Division in response to a request from the Office of Water Supply.

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

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METHODS FOR THE DETERMINATION OF CHEMICAL CONTAMINANTS IN DRINKING WATER

INTRODUCTION

Course Instructional Objectives:

This course is designed to meet the needs for training those persons who will be involved in analysis of potable waters for contaminants listed in the Interim Primary Drinking Water Regulations. The course can be used by either State or Federal personnel. Should the course be offered by a State, the contents should be thoroughly checked to see if discrepancies exist between the outlines prepared under the Federal regulations and what is required in the State's Drinking Water Act.

After successfully completing this course, the trainee should have sufficient information to carry out the various analyses. The trainee will perform analysis in the laboratory under the supervision of the offering authority's chemist. The trainee will be observed by the instructors and judged on the competence of laboratory technique and will be given a test upon completion of the course.

The course will cover the analytical methodology used to analyze for metals, organics, nitrates and fluoride. Additional topics covered will include sample handling and discussions on laboratory certification and the Act itself.

Although all parameters, inorganic and organic, are contained in this manual, caution is advised in several areas. First there is a provision in all acts, both Federal and State, to allow alternate test procedures. This provision can be carried out again on a Federal or State level. Consequently, the users of this manual should inquire of their appropriate authority as to the existance of any additional methodology which has been approved.

Secondly, from time to time the Primary Drinking Water Regulations will be amended and new parameters which must be analyzed or added. This will create a need for constant revision of the manual on the part of the user to keep it current. Another thought should be considered here and that is the Secondary Regulations. These will be issued on asthetic principles and will not be mandatory. However, the methodology that should be used is listed in these regulations and analysts might wish to examine these.

The exact make-up of the manual has been designed in such a way as to allow maximum flexibility in determination of course content. Options have been provided so as to allow the course director to select the final content.

For a brief overview of the course, the content covered, and the schedule, turn to the Agenda.

A word about the handbook itself is in order. The handbook is designed to be used by you from the time you receive it throughout the course, and when you are back on the job. At the outset, there are some tasks the participant should complete prior to attending the course. During the course, the handbook will be referred to daily or with each set of units and presentations. It spells out for you what you will learn, how, when, under what conditions, and when you will know if you have learned what is intended. The instructors will use this same material, with some variations depending on the situation, participant needs, or scheduling requirements.

The handbook is assembled in this loose-leaf form to allow the instructor some flexibility in unit selection or sequencing. Also, it allows you to insert your notes from the daily sessions next to the corresponding unit. Should the instructor or a fellow-participant provide additional information (a paper, articles, etc.) not envisioned for the course but relevant and useful, it can be inserted in the handbook.

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METHODS FOR THE DETERMINATION OF CHEMICAL CONTAMINANTS IN DRINKING WATER

PRE-COURSE ACTIVITIES

Each of the following forms to be completed by you concern activities you are asked to complete prior to the course itself. Those are the Pre-test, Biographic Statement, and Participant Survey. They are explained in detail in the introduction to each form.

A fourth sub-section is entitled Pre-Course Preparation. It is simply those materials or activities the instructor may appropriately deem important to read or carry out prior to arriving for the course. This part of the handbook is optional and for the instructor to include or exclude.

______________________________ Course Pre-Test

Dear Participant:

As your first action in relation to this course, we are asking you to complete a short pre-test and return it to us before coming to the course. It is important to have this information in order to measure how effectively the course is meeting its objectives. This, plus the post-test given upon completing the course, will help us with this measurement.

Note, we talk of "measuring the course," not measuring you in the sense of pass or fail. While learning is the primary responsibility of the learner, we recognize that course design, content, methodology, instructor, learning environment, etc., all play a role. This measurement plus other feedback the instructors will get, will assist us with future design and delivery.

You are encouraged to take this test without any outside help from books or individuals. This test is designed to answer only the question of have you improved your knowledge by taking this course. It will also give some indication on course design and instructor teaching. No grade will be assigned nor is any other use intended.

Pre-Test

1. The analytical methods to be used in the analyses of Drinking Water Samples are set down in the

   a. Interim Primary Drinking Water Regulations
   b. Safe Drinking Water Act
   c. Clean Water Act

2. Organic samples are collected in containers.

   a. glass or plastic
   b. glass only
   c. plastic only

3. The maximum holding time recommended for metals is

   a. 14 days
   b. 180 days
   c. 30 days

4. A statistical measurement for precision is

   a. percent recovery
   b. central tendency
   c. standard deviation

5. Minimum quality control requires that daily checks of a standard curve be within of the original curve.

   a. ± 10%
   b. ± 5%
   c. ± 2%

6. Safety practices should be carried out

   a. at all times
   b. only when hazardous materials are being used
   c. only when the supervisors are watching

7. The required analytical method, published in the Interim Primary Regulations, for silver is

   a. dithizone
   b. silver diethyldithiocarbamate
   c. standard atomic absorption techniques

8. The MCL for silver was based on considerations.

   a. cost
   b. aesthetic
   c. health

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9. The oxidant and fuel gases used when silver is determined are

   a. air-acetylene
   b. nitrous oxide-acetylene
   c. argon-hydrogen

10. In order to determine cadmium, chromium and lead at their MCL's, the sample needs to

   a. be solubilized
   b. extracted and concentrated
   c. both

11. The oxidant and fuel gases used to determine cadmium, chromium and lead are

   a. air-acetylene
   b. nitrous oxide-acetylene
   c. argon-hydrogen

12. The extraction technique

   a. necessitates doing each metal (cadmium, chromium, lead) separately
   b. allows all metals (cadmium, chromium, lead) to be done with one extraction
   c. need a preliminary colorimetric procedure.

13. The determination of mercury is carried out by

   a. normal atomic absorption techniques
   b. colorimetricly
   c. a flameless atomic absorption technique

14. The oxidant and fuel gases used to determine mercury

   a. are air-acetylene
   b. nitrous oxide-acetylene
   c. air only

15. The organic forms of mercury are ______ to convert to metallic form.

   a. difficult
   b. easy
   c. impossible

16. The oxidant and fuel gases used to determine arsenic and selenium are

   a. air-acetylene
   b. nitrous oxide-acetylene
   c. argon-hydrogen

17. Organic forms of arsenic are analyzed ______ by the gaseous hydroxide method.

   a. directly
   b. after an oxidation step
   c. colorimetricly

18. The zinc slurry provides in the gaseous hydride procedures for arsenic and selenium.

a. for the reduction
b. the hydrogen for the flame
c. the hydrogen to form the hydride

19. Nitrate is determined in the cadmium reduction method.

a. as nitrate
b. as nitrite
c. as cadmium

20. Nitrate samples for the reduction column should not be preserved with

a. sulfuric acid
b. refrigeration at 4°C
c. mercuric chloride

21. The nitrate sample for the cadmium reduction method is filtered to remove turbidity which could

a. react with the nitrate
b. oxidize the nitrate to nitrite
c. restrict flow through the column

22. The brucine test analyzes nitrate as

a. nitrate
b. nitrite
c. brucine

23. One extremely important control in the brucine test is

a. size of the particles
b. concentration of nitrite
c. temperature

24. The brucine-nitrate test is a test.

a. colorimetric
b. atomic abosrption
c. titrametric

25. For drinking water samples must precede the SPADNS test.

a. filtration
b. use of the electrode
c. distillation

26. Fluoride samples are preserved by the addition of

a. nothing
b. nitric acid
c. mercuric chloride

27. The SPADNS method for fluoride is a   procedure.

    a. colorimetric
    b. atomic absorption
    c. titrametric

28. The distillation procedure works by

    a. distilling over the interferences and leaving the F^- behind
    b. distilling over the F^- and leaving the interferences behind
    c. forming a color with the interferences

29. A new batch of acid/water mix must be used

    a. with each sample
    b. after three samples
    c. when the solution turns brown

30. If the temperature is allowed to go beyond 180°C

    a. the fluoride is not distilled
    b. the iron carries over
    c. sulfate is carried over

31. For drinking water samples   must procede the electrode method.

    a. nothing
    b. distillation
    c. filtration

32. The electrode must be connected to   for the F^- determination.

    a. pH meter with expanded scales
    b. specific ion meter
    c. either of the above

33. The electrode itself and the   account for the small number of interferences.

    a. distillation
    b. the TISAB buffer
    c. the complexone

34. Barium is determined by

    a. colorimetry
    b. atomic absorption
    c. titrametry

35. The barium samples are preserved by adding

    a. nitric acid
    b. sulfuric acid
    c. mercuric chloride

36. In order to express the value of "total" barium, a step must be performed.

    a. filtration
    b. weighing
    c. solubilization or digestion

37. The approved method for residual chlorine determination for water supply samples is the

    a. o-tolidine
    b. phenol red
    c. DPD

38. The kit form of the approved method

    a. can be used
    b. cannot be used
    c. must be applied for under alternate test procedures

39. Chlorine samples

    a. can be preserved overnight
    b. can be held for 48 hours
    c. cannot be preserved

40. The turbidity sample must be taken

    a. in the plant
    b. at an entry point to the distribution system
    c. in the distribution system

41. The reason for the MCL on turbidity is because

    a. it may interfere with disinfection
    b. it makes water look bad
    c. it makes water taste bad

42. Turbidity measurement must be carried out

    a. a number of times based on population served
    b. once a week
    c. once a day

43. The Interim Primary Drinking Water Regulations

    a. become effective in December of 1977
    b. became effective in December of 1975
    c. became effective in June of 1977

44. The Interim Primary Drinking Water Regulations include maximum contaminant levels (MCL's) as well as monitoring frequencies for

    a. chemical, bacteriological, radiological contaminants
    b. chemical, bacteriological contaminants
    c. chemical contaminants

45. The maximum holding time for the chlorinated hydrocarbons samples is

   a. 14 days
   b. 7 days
   c. none

46. The pesticides which are to be monitored are

   a. endrin, aldrin, lindane, methoxychlor, toxaphene
   b. endrin, lindane, methoxychlor, aldrin
   c. toxaphene, methoxychlor, lindane, endrin

47. The pesticides are extracted from the sample using

   a. hexane
   b. petroleum ether-ethylether
   c. hexane-methylene chloride

48. The chlorphenoxy herbicides to be monitored for are

   a. 2,4, D; 2,4,5 TP; 2,4,5 T
   b. 2,4, D; 2,4,5 T
   c. 2,4, D; 2,4, 5 TP

49. The herbicides are extracted from the sample using

   a. hexane
   b. hexane-methylene chloride
   c. ethylether

50. The maximum holding time for herbicide samples is

   a. 14 days
   b. 7 days
   c. none

BIOGRAPHIC STATEMENT

Dear Participant:

We ask that you answer and complete the following items as briefly as possible. The purpose is simple: to acquaint the instructor with his/her students prior to the course.

1) Name: (please print)

2) Date:

3) Address:

4) Date of Birth:

5) Present Position or Job Title:

6) Major job functions or responsibilities:

7) Courses or study undertaken in relation to job:

PARTICIPANT SURVEY FOR THE METHODS OF ANALYSIS FOR INORGANIC CONTAMINANTS OF POTABLE WATERS

1. Identify the experience you have with each method for the various contaminants covered in the course (give approximate length of experience).

2. How much experience do you have in atomic absorption?

3. Do you have sufficient basic laboratory skills to carry out necessary laboratory procedures (use student skills checklist)?

4. List the goals which you wish to achieve in attending this course.

5. Will there be a need for you to pass on the information attained in this course to others?

Name ______________________________

Employer ____________________________

STUDENT SKILLS CHECKLIST

To assist us in processing applications, please check YES or NO for each of the following items:

	YES	NO
I have operated a laboratory gas burner		
I have operated a laboratory hotplate/stirrer		
I have operated an autoclave		
I have operated a laboratory drying oven		
I have used a vacuum source to filter liquids		
I have used a desiccator		
I have weighed items on an analytical balance		
I have weighed items on a double pan balance		
I have used a graduate to measure liquids		
I have used a volumetric pipet to measure liquids		
I have used a graduated pipet to measure liquids		
I have used a pipet bulb to fill a pipet		
I have used mouth suction to fill a pipet		
I have used an inoculating loop to transfer small amounts of liquid		
I have used disinfectant to sterilize a lab bench work area		
I have poured liquid from a container into glass test tubes		
I have prepared media used for coliform tests		
I have used chromic acid to clean glassware		
I have operated a laboratory safety shower		
I have operated a laboratory eye washer		
I have operated a fume hood		
I have prepared manganous sulfate solution		
I have made out labels for bottles or reagents		
I have used a buret		
I have used starch as a chemical change indicator		
I have titrated one solution against another to a color change end Point		
I have recorded a reading at a meniscus		
I have recorded laboratory data in a laboratory notebook		
I have entered laboratory data on a pre-printed form		
I have recorded information about samples on record sheets		
I have located required purchase information in a catalog of laboratory equipment		
I have written a purchase order for chemicals to be used in the lab		

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Name ____________________________

Employer __________________________

	YES	NO
Volume means space occupied by a solid, liquid, or gas	____	____
mg/l means milligrams per liter	____	____
Normality (N) is a way to express concentration in a solution . .	____	____
1 kilogram equals 0.001 gram	____	____
1 inch equals 2.54 cm	____	____
1000 ml equals 1 liter	____	____
85 times 4.1 equals 42.5	____	____
7 minus 2 divided by 0.02 equals 250	____	____
3.26 rounded to the nearest tenth is 32.6	____	____
84.55147 rounded to the nearest thousandth is 84.551	____	____

INSTRUCTIONAL UNITS

INTRODUCTION

The material covered in this section represents the core of the course. All the parameters listed in the Interim Primary Regulations are covered. Where more than one analytical procedure has been permitted, all are included here. The analyst will have to make the choice as to which will be used. The material has been written in a format that allows the trainee to utilize the outline as the analysis is carried out. The step-by-step outline proceeds in the same order as an analyst must proceed. Each step is given and if warranted, additional information is given in the next column. The student should be cautioned to read each step and the material, if any, in the information column before doing the step.

Also included in the outline is an equipment list which lists capital, reuseable and consumable items necessary to perform the analysis. Where more than one analysis is being performed, the equipment lists will have duplication and the trainee must purchase only what is needed.

The level of training required to perform each analysis varies. For example, the outlines on residual chlorine and turbidity need little formal chemical educational background to be performed. However, the atomic absorption and gas chromatographic analysis require considerable background to perform. The later two analyses should be performed only by experienced chemists or done under their supervision.

As new persons enter into the laboratory and assume responsibility for certain analyses, this manual or sections of it can be used to acquaint or refresh the analyst with the methodology for which he is responsible.

REGULATIONS CONNECTED WITH THE SAFE DRINKING WATER ACT

I. Introduction

The primary interest of the Safe Drinking Water Act was to produce potable water for the consuming public. It is estimated that there are 240,000 water supplies in the United States. That is about 40,000 community supplies and about 200,000 non-community water supplies. The Safe Drinking Water Act required that limits be set down for those materials which, when found in natural waters, would pose a health hazard. From this requirement a document was drawn up and published for public comment. This was the Proposed Interim Primary Regulations. After the comments were received and acted upon, the Interim Primary Regulations were published in the Federal Register. These Interim Primary Regulations listed the health related agents to be monitored for in the nations drinking waters. A monitoring frequency was set down for the community and non-community supplies and a "Maximum Contaminant Level" (MCL) for each was set. In addition, a list of "approved" methods for the analysis of each parameter was given.

The "Act" stipulated that a study was to be made by the National Academy of Science (NAS) on the parameters that were included in the Interim Primary Regulations. This study was to look at other compounds to include, if some parameters should be excluded, if the MCL were right and suggest changes and areas of research. After the NAS report was finished the Interim Primary Regulations were to be revised and published for public comment and then promulgated.

The Environmental Protection Agency was to publish a list of parameters and methods to analyze for these parameters which a treatment facility might wish to monitor for. These parameters were to be based on aesthetic values and were not to be of an enforceable nature. These "Secondary Regulations" were to follow the route of the Primary in that they were to be proposed and sent out for public comment.

In addition the the Primary regulations, EPA was called on to issue any additional parameter it felt might endanger the health of the consuming public. These additional parameters after being commented on would be a part of the already published Primary Regulations. As with the other parameters analytical methodology and monitoring frequencies would be published for the new parameters.

After publication of the analytical methodology in the Primary Regulations, any new methods could be used after being ajudged comparable to those already published. A review panel and an approved mechanism was to be set up to supervise the requests for "Alternate Test Procedure Approval." If the new method was found to be comparable, it could be approved on a national or regional area level.

II. National Interim Primary Drinking Water Regulations

Section 1412 of the Safe Drinking Water Act requires the Environmental Protection Agency to publish proposed national interim primary drinking water regulations. These regulations were to be proposed 90 days after the enactment of the Safe Drinking Water Act. Public comments were to be solicited on the proposed Interim Primary Drinking Water Regulation and these regulations were to be promulgated 180 days after enactment.

The Proposed Interim Regulations were issued on March 14, 1975, and promulgated on December 24, 1975. Since the Act states that these regulations should become effective 18 months after promulgation, they became effective June 24, 1977. These Interim Primary Regulations will be revised on an as needed basis as well as at least every three years.

Since the Interim Primary Regulations are based on health hazards to the consuming public, these regulations contain a list of Organic and Inorganic materials and a maximum contaminant level for each. These levels are based upon possible health hazards that may occur after a lifetime of exposure of approximately two liters of water per day. The regulations set down what must be done by a water supply should one of these maximum contaminant levels (MCL) be surpassed. Also, a part of the regulations is a list of approved methods for the analysis of the parameters listed. The parameters, their levels and methods are listed in Table I.

Also included in the regulations are the sampling and monitoring frequencies for the published parameters. This sets down how often samples must be analyzed and for which of the parameters. Microbiological parameters are also listed in the Interim Regulations. The limits and sampling frequencies are covered in other manuals. The chemical sampling and monitoring requirements are listed in Table 3.

Under the microbiological sections in the Interim Primary Regulations a supply may substitute residual chlorine determinations for a portion of the microbiological tests. The level of free chlorine that must be maintained is 0.2 mg/l. The analytical method to determine this is the DPD colorimetric or titrimetric method. It was the intent to allow the use of the color comparator kits for this analysis. Due to the inability to preserve a free chlorine sample it is expected that the operator himself will perform this test.

III. National Academy of Science Study

After publication of the Proposed and Interim Primary Regulations the EPA is required by the Act to enter into arrangements with the National Academy of Sciences to conduct a study. This study should determine the maximum contaminant levels which should be recommended under the revised primary regulations in order to protect the health of persons from any known or anticipated adverse effects, and the existence of any contaminants - the levels of which in drinking water cannot be determined but which may have an adverse effect on the health of persons.

The study was to be presented to Congress no later than 2 years after the date of enactment of the Act. EPA will use this study in deciding whether to include any such contaminants in a revised Primary Drinking Water Regulations.

In conducting its study the National Academy of Sciences is directed to consider only what is required to protect public health, not what is technologically or economically feasible or reasonable. Based on the results of the NAS study, EPA may specify additional contaminants with adverse health effects. It may establish new maximum contaminant levels. It may prescribe a list of known water treatment techniques which will reduce the concentration of any contaminant for which no maximum contaminant level is established (e.g., viruses, organics, asbestos), or it may establish requirements for operation and maintenance.

TABLE 1

Parameter	Limit mg/liter	Method	Reference	
Inorganic - Other than Fluoride			EPA[1]	Std. Method[2] 13th
Arsenic	0.05	Atomic Absorption (gaseous hydride)	95-96	
Barium	1.00	Atomic Abosrption (Std. Conditions)	97-98	210-215
Cadmium	0.01	Atomic Absorption (Std. Conditons)	101-103	210-215
Chromium	0.05	Atomic Absorption with Chelation Ext.	105-106	210-215
Lead	0.05	Atomic Absorption with Chelation Ext.	112-113	210-215
Mercury	0.002	Flameless Atomic Absorption	118-126	
Nitrate	10.	Brucine (Colorimetric) Cadmium Reduction (Colorimetric)	201-206	461-464
Selenium	0.01	Atomic Absorption (gaseous hydride)	145	
Silver	0.05	Atomic Absorption (Std. Conditions)	146	210-215
Turbidity	1.0 TU	Nephelometric	295-298	350-353
Fluoride	Temp. Dep. See Table 2	Electrode	65-67	172-174
		SPADNS (Colorimetric) with distillation	59-60	171-172 174-176

Parameter	Limit mg/liter	Method	Reference
Organic			
Chlorinated Hydrocarbons		Gas Chromatography	EPA Method [3]
Endrin	0.0002		
Lindane	0.004		
Methoxychlor	0.1		
Toxaphene	0.005		
Chlorophenoxys		Gas Chromatography	EPA Method [4]
2.4-D	0.1		
Silvex	0.01		

[1] Methods for Chemical Analysis of Water and Wastes, EPA, Office of Technology Transfer, Cincinnati, Ohio 45268.

[2] Standard Mehtods for the Examination of Water and Wastewater, 13th Edition, 1971, APHA, 1015 18th St., NW, Washington, DC 20036.

[3] Method for Organochlorine Pesticides in Industrial Effluents, EMSL, EPA, Cincinnati, Ohio 45268.

[4] Method for Chlorinated Phenoxy Acid Herbicides in Industrial Effluents, EMSL, EPA, Cincinnati, Ohio 45268.

TABLE 2

Fluoride MCL

Temperature °C	MCL mg/l
12.0 and below	2.4
12.1 to 14.6	2.2
14.7 to 17.6	2.0
17.7 to 21.4	1.8
21.5 to 26.2	1.6
26.2 to 32.5	1.4

TABLE 3

Public Water Supply Sampling Requirements

Type of Contaminant	Water Source	Sampling Period Initated by	Sampling Period Conclude By	Sampling Frequency Thereafter	If MCL is Exceeded
		Community Water Supplies			
Inorganic	Surface	6/24/77	6/24/78	at yearly intervals	collect 3 additional samples in one month
	Ground	6/24/77	6/24/79	every three years	
Organic	Surface	6/24/77	6/24/78	every three years or as required by State	"
	Ground	To be specified by State		To be specified by State	
Turbidity	Surface	6/24/77	---	must measure daily	resample within one hour
		Non-Community Water Supplies			
Inorganic-Nitrate only	Surface and Ground	6/24/77	6/24/79	To be specified by State	collect 3 additional samples in one month
Turbidity	Surface	12/24/77	---	must be measured daily	resample within one hour

Report to State any violations within 48 hours, including violation of monitoring requirements.

Report to State tests results within 40 days.

IV. Proposed National Secondary Drinking Water Regulations

The Safe Drinking Water Act, Section 1412 C, required the EPA to propose national secondary drinking water regulations. These regulations were to be propsoed within 270 days after the enactment of the Safe Drinking Water Act. Again, public comment was to be requested and acted upon before promulgation of the secondary regulations. These secondary regulations are to be based on contaminants that tend to make water disagreeable to use, but that do not have any particular adverse public health effect.

The secondary regulations were proposed on March 31, 1977, and included the following:

TABLE 4

Contaminant	Limit	Method	Reference EPA	Reference 14th Std. Meth.
Chloride	250 mg/l	Potentiometric		306
Color	15 Color Units	Platinum-Cobalt	36-38	64
Copper	1.0 mg/l	Atomic Absorption	108-109	144
Corrosivity	Non-Corrosive	Reserved		
Foaming Agent	0.5 mg/l	Methylene Blue	157-158	600
Hydrogen Sulfide	0.5 mg/l	Titrimetric-Iodine	284	505
Iron	0.3 mg/l	Atomic Absorption	110-111	144
Manganese	0.05 mg/l	Atomic Absorption	116-117	144
Odor	3 Threshold Odor Number	Consistent Series	287-294	75
pH	6.5 - 8.5	Glass Electrode	239-240	460-465
Sulfate	250 mg/l	Turbidimetric	277-278	496
TDS	500 mg/l	Total Residue	270-271	91
Zinc	5 mg/l	Atomic Absorption	155-156	144

These secondary regulations are applicable to all public water systems, but are not enforceable on the Federal level and are intended as guidelines for the states. However, depending on their legislation, may be enforceable by the State.

V. Alternate Test Procedures Approval

Section 141.27 of the National Interim Primary Drinking Water Regulations permitted the establishment of a procedure for Approval of Alternate Analytical Methods. On March 10, 1977, this procedure was established. Two parallel approval chains are allowed, a case by case procedure and a procedure for national approval. Alternative analytical techniques approved for nationwide use will be published as amendments to the Interim Primary Regulations in the Federal Register.

Several alternate methods have been approved for nationwide use, but have not yet been published in the Federal Register. These are as follows:

Measurement	Method
Arsenic	[1] Flameless Atomic Absorption, Graphite Furnace Technique.
Arsenic	Silver Diethyldithiocarbamate Method, Ref: "Methods for Chemical Analysis of Water and Wastes," pp. 9-10, EPA, Office of Technology Transfer, 1974.
Barium	[1] Flameless Atomic Absorption, Graphite Furnace Technique.
Cadmium	[1] Flameless Atomic Absorption, Graphite Furnace Technique.
Chromium	[1] Flameless Atomic Absorption, Graphite Furnace Technique.
Fluoride	Automated Alizarin Fluoride Blue, Ref: "Standard Methods for the Examination of Water and Wastewater," 14th, pp. 614-616, 1975.
Fluoride	[2] Zirconium-Eriochrome Cyanine R, Ref: "Methods for Collection and Analysis of Water Samples for Dissolved Minerals and Gases," USGS, Book 5, Chapter A 1, pp. 90-93.
Lead	[1] Flameless Atomic Absorption, Graphite Furnace Technique.
Mercury	Automated Cold Vapor Technique, Ref: "Methods for Chemical Analysis of Water and Wastes," pp. 127-133, EPA, Office of Technology Transfer, 1974.
Nitrate	Automated Hydrazine Reduction, Ref: "Methods for Chemical Analysis of Water and Wastes," pp. 185-194, NERC, Analytical Quality Control Laboratory, 1971.

Measurement	Method
Nitrate	Automated Cadmium Reduction, Ref: "Methods for Chemical Analysis of Water and Wastes," pp. 207-212, EPA, Office of Technology Transfer, 1974.
Organics	[2] Gas Chromatographic, Ref: "Methods for Analysis of Organic Substances in Water," USGS, Book 5, Chapter A 3, pp. 24-39.
Selenium	[2] Hydride generation - atomic absorption spectrophotometry, USGS,. Method, I-1667-77, 1976.
Selenium	Flameless Atomic Abosrption, Graphite Furnace Technique, Ref: Atomic Absorption Newsletter, 14, No. 5, pp. 100-116, 1975.
Silver	[1] Flameless Atomic Absorption, Graphite Furnace Technique.

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[1] The various furnace devices are considered to be atomic absorption techniques. Methods of standard addition are to be followed as noted on p. 78 of "Methods for Chemical Analysis of Water and Wastes," EPA, Office of Technology Transfer, 1974.

[2] Copies available from: Water Quality Branch, National Center, U. S. Geological Survey, 112201 Sunrise Valley Drive, Reston, VA 22092.

[3] Only the six pesticides named in the Interim Primary Drinking Water Regulations are included: Endrin, Lindane, Methoxychlor, Toxaphene; 2,4-D; and 2,4,5-TP (Silvex). Federal Register, Vol. 40, No. 248, pp. 59570-59571, Dec. 24, 1975.

VI. Certification

Section 1401 (1) of the Safe Drinking Water Act defines "Primary Drinking Water Regulations" to include "quality control and testing procedures" to insure compliance with maximum contaminant levels. Pursuant to the Act, the National Interim Primary Drinking Water Regulations, Section 141 and 142 require that for compliance purposes, "samples" will be considered only if they have been analyzed by a laboratory approved by the State, except that measurement for turbidity and free chlorine residual may be performed by any person acceptable to the State, and the State must establish and maintain a program for certification of laboratories conducting measurement of drinking water contaminants.

A "Manual for the Interim Certification of Laboratories Involved in Analyzing Public Drinking Water Supplies" has been compiled. This manual describes how the Environmental Protection Agency will carry out a tentative program for interim approval and certification of its ten Regional laboratories and principal State laboratories. States without certification programs are encouraged to use this program as a model; States with equivalent or better certification programs are encouraged to continue and improve.

The manual describes evaluation procedures and minimum technical requirements recommended for certifying laboratories analyzing public drinking water supplies. In addition to identifying requirements that are critical to generation of valid data, optional certification requirements have been included as guidance.

SAMPLING

I. INTRODUCTION

With the intent of the Safe Drinking Water Act being the insurance of proper drinking water quality, meaningful analysis of the water is imperative to know if the water meets the standards. This analysis can only be meaningful if it is performed on a sample that is representative of the water to be analyzed. Consequently, the proper sampling technique, use of proper containers, proper preservation and adherence to the set frequency of sampling must be carefully observed.

In many instances the laboratories themselves will not be responsible for sampling. However, it is necessary that all laboratories be aware of what constitutes a representative, properly taken sample. It is the responsibility of all laboratories sampling for parameters under the Safe Drinking Water Act to call for a resample if the sample does not meet proper sampling procedures. To analyze a sample which has been doubtfully taken is to present data which is dubious in meaning. If the laboratory is responsible for taking the samples, it is doubly important that the persons in the laboratory be aware of proper techniques.

There is at this time under development a "Handbook for Sampling and Sample Preservation of Water and Wastewater" by the U.S. Environmental Protection Agency. When this book is available it will serve as a good reference source on the topic of sampling.

Section F of the Criteria and Procedures document for Laboratory Certification spells out the mandatory requirements that must be adhered to for the drinking water sampling. It is attached here for student reference as this outline is read.

It has been suggested that one of the certification team members be from the staff of the regional water supply staff. This individual would be responsible to assess the following:

A. Choice of sampling location
B. Proper sampling procedures
C. Sample identification
D. Prompt sample transport to the laboratory
E. Sampling frequency
F. Bad-sample follow-up
G. Dissemination of data by the laboratory and use of the data by the water supply supervision program

II. MONITORING REQUIREMENTS

A. Inorganic

1. Analysis for all community water systems utilizing surface water sources shall be completed within one year following June 1977. These analyses shall be repeated at yearly intervals.

2. Analysis for all community water systems utilizing only ground water sources shall be completed within two years of June 1977. These analyses shall be repeated at three year intervals.

3. For non-community water systems, whether supplied by surface or ground water sources, analysis for nitrate shall be completed within two years of June 1977. These analyses shall be repeated at intervals determined by the state or other regulatory agency.

Sample collecting, handling, and preservation[4] -- mandatory requirements

Parameter	Preservative	Container (1)	Maximum holding time (2)
Arsenic	Conc HNO_3 to pH<2	P or G	6 months
Barium	Conc HNO_3 to pH<2	P or G	6 months
Cadmium	Conc HNO_3 to pH<2	P or G	6 months
Chromium	Conc HNO_3 to pH<2	P or G	6 months
Lead	Conc HNO_3 to pH<2	P or G	6 months
Mercury	Conc HNO_3 to pH<2	G P	38 days 14 days
Nitrate	Conc H_2SO_4 to pH<2	P or G	14 days
Selenium	Conc HNO_3 to pH<2	P or G	6 months
Silver	Conc HNO_3 to pH<2	P or G	6 months
Fluoride	None	P or G	1 month
Chlorinated hydrocarbons	Refrigerate at 4°C as soon as possible after collection	G with foil or Teflon-lined cap	14 days (3)
Chlorophenoxys	Refrigerate at 4°C as soon as possible after collection	G with foil or Teflon-lined cap	7 days (3)

(1) P = Plastic, hard or soft, G = Glass, hard or soft

(2) In all cases, samples should be analyzed as soon after collection as possible.

(3) Well-stoppered and refrigerated extracts can be held up to 30 days.

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[4] If a laboratory has no control over these factors, the laboratory director must reject any samples not meeting these criteria and so notify the authority requesting the analyses.

[5] If nitric acid cannot be used because of shipping restrictions, sample may be preserved by icing. Upon receipt in the lab, the sample must be acidified with conc HNO_3 to pH<2. At time of analysis, sample container should be thoroughly rinsed with 1:1 nitric acid washings added to the sample to be processed for subsequent analysis.

4. When the maximum contaminant level is surpassed, the frequency of resample shall be designated by the state and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

B. Organic

1. For all community water systems utilizing surface water sources, analysis shall be completed within one year of June 1977. Samples analyzed shall be collected during the period of the year designated by the state as the period when contamination by pesticides is most likely to occur. These analysis shall be repeated at intervals specified by the State, but in no less frequency than at three year intervals.

2. For community water systems utilizing only ground water sources, analysis shall be completed by those systems specified by the State.

3. If the results of an analysis indicate that the level of any contaminant exceeds the maximum contaminant level, the supplier of water shall report to the State within 7 days and initiate three additional analyses within one month.

4. When the average of four analyses exceeds the maximum contaminant level the supplier of water shall report to the State. Monitoring after public notification shall be at a frequency designated by the State and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

III. SAMPLE CONTAINERS

A. Types

Generally two types of containers are acceptable; these are glass and plastic. Plastic is the more convenient from a shipping standpoint; however, plastic may not be used for the organic parameters. The glass containers should preferably be made from a hard borosilicate glass (Kimax or Pyrex; however, other forms may be used).

All these various materials have certain advantages and disadvantages. The hard glass is inert to most materials. Conventional polyethylene is to be used when plastic is acceptable because of reasonable cost and less adsorption. Disposable type plastic containers, such as the molded polyethylene "Cubitainer," are convenient to use.

Usually, a wide mouth container is preferred. This allows easy sample removal and easier cleaning.

Depending on the State or Regional requirements, three or four containers will be needed for a complete analysis. Usually, a one gallon or equivalent size plastic type container will suffice for the metals analyses. A one quart or equivalent plastic container is needed for the nitrate sample. A glass container holding about a quart with a foil or Teflon lined screw cap is required for analyses of the organics, and finally, a one quart plastic type container will be needed for the fluoride parameter.

Considering the section on frequency of monitoring, the following are needed for each public water supply in one years time:

1. One 1 gallon plastic container

2. Two 1 quart plastic container

3. One 1 quart glass container

Additional containers would be needed for resampling needs. A non-community supply would probably need one 1 quart plastic container each year unless resampling is to be carried out.

In addition to the containers themselves, some type of shipping container must be provided for each sample container. These shipping containers can usually be purchased from the supplier of the actual sample container.

B. Preparation and Shipment of Containers

Individual responsibility to provide, maintain and clean sample containers is dependent on how the State has elected to carry out the certification program. The laboratory or the authority could purchase in large lots and make available sets of containers to each supply or the State may elect to require the supply to provide their own containers. Generally speaking, the plastic containers should not be reused for any trace analyses, that is, the metals. The glass containers should follow the suggested cleaning procedure, including muffling at 400°C for about 15 minutes. Once cleaned, these containers should be stored and shipped in such a manner as to prevent recontamination.

Should the decision to reuse plastic containers be made, they should be cleaned carefully before reuse. There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

1. Traces of dichromate cleaning solution will interfere with metal analyses. Use 1:1 nitric acid wash.

2. Traces of nitric acid may interfere with the nitrate analysis. Use detergent with thorough rinses with tap and distilled water.

Shipping the containers to the sampling locations should take into consideration the numbers to be shipped and eliminate any contamination chances. The shipping containers to be used in transporting the sample itself to the laboratory must be provided either as a container for the empty sample container or in bulk form.

One item that must be given consideration is the preservative. Postal regulations will not permit mailing of acids, particularly nitric acid. Consequently, these materials must be purchased locally or shipped by truck or other common carrier. If the materials are to be purchased locally, the purity must be rigidly controlled to assure no contaminants are present to effect results.

Even when the preservative nitric acid has been added and diluted by the sample, postal restrictions may preclude the use of the mails. Therefore, a special footnote has been added to the certification procedures allowing an alternate icing followed by acidification upon receipt of the sample in the laboratory.

When shipping the sample to the laboratory for analysis, sufficient time should be allowed to assure that the holding times are not surpassed. Alternate forms of transportation should be checked out beforehand to allow use if needed. The sample container must be protected from physical damage in shipment and sufficient coolant added to the ice chest or other form of insulated container to last through the duration of shipment. Caps should be checked when the sample is taken to assure that they will not leak. Upon receipt in the laboratory, any deviation from the mandatory sampling requirements, i.e., preservative, holding times, should be noted and, if necessary, a resample ordered immediately.

IV. SAMPLE COLLECTING

According to the National Interim Primary Drinking Water Regulations, section 141.2(c), the sampling location is the "free-flowing outlet of the ultimate consumer." Since this represents a minimal effort, one sample can be taken at any point in the distribution system and fulfill the regulation. Some States may require more frequent samples at random locations, or a single composite sample taken at various locations.

The exception to this sampling location is the turbidity sample which must be taken at the point of entry of the water into the distribution system.

When collecting the sample, the tap should be run to assure that the water collected is from the distribution system and not from the private pipes. The sample container should be flushed two or three times before the actual sample is taken. The container should not be filled completely to allow extra volume for effects of temperature during transit. The preservative, if any, should be carefully added to the container, the container capped and the sample shaken.

If the sample is to be cooled during shipment, the sample container should be placed in an insulated container and sufficient coolant added to last during shipment.

The sample should be labeled to identify it during future analyses. The information should include:

A. Date, place and time of sampling; name of person collecting the sample.

B. Identification of the sample as to whether it is a routine distribution system sample, check sample, raw or process water sample, or other special purpose sample.

C. Analysis to be run on the sample as well as any preservative added and what amount has been added.

D. Any other remarks that the sampler thinks is necessary.

This information should be affixed to the sample container in such a way as to assure that it will not become separated in later handlings.

The Criteria and Procedures Document for Laboratory Certification states that chain of custody procedures must be carried out on all samples taken for potential enforcement actions only. The exact procedure and directions on this procedure should be obtained from the appropriate certification authority.

V. FIELD MEASUREMENTS

As set down in the act, there are two types of analysis which may be carried out in other than certified laboratories. These are the analysis for residual chlorine and turbidity. These measurements may be carried out in the field. In addition, should any other information about the sample be required, such as pH, temperature, etc., these should also be carried out in the field. It is not the scope of this outline to discuss the procedures involved in these analyses. Procedures on the residual chlorine and turbidity have been included in this manual for information purposes only and may be found under Tab F.

State regulations may require additional procedures to be carried out by the person taking the sample. The Interim Primary Regulations do not.

VI. SUMMARY

The importance of proper sampling is the foundation of meaningful analytical results. Consequently, a laboratory should know what constitutes a meaningful sample inorder to judge when a resample is necessary due to improper sampling, preservation or handling techniques.

The preservative to be added, the type of container and the holding times are spelled out in the Criteria and Procedures Document in a mandatory section.

The laboratory Certification Officer must evaluate whether or not the laboratory is conducting a proper sample receipt procedure and, if it has the responsibility, a proper sampling of the water supplies.

STATISTICS FOR CHEMISTS

I INTRODUCTION

A Statistics may be defined, for our purpose, as a collection of methods which have been developed for handling numerical data pertaining to samples or portions of entire populations.

B The statistical methods with which we will concern ourselves deal with the presentation and analysis of numerical data from samples.

II FREQUENCY

A Definitions

1 Frequency - indicates how many times a particular score occurs in a collection of data

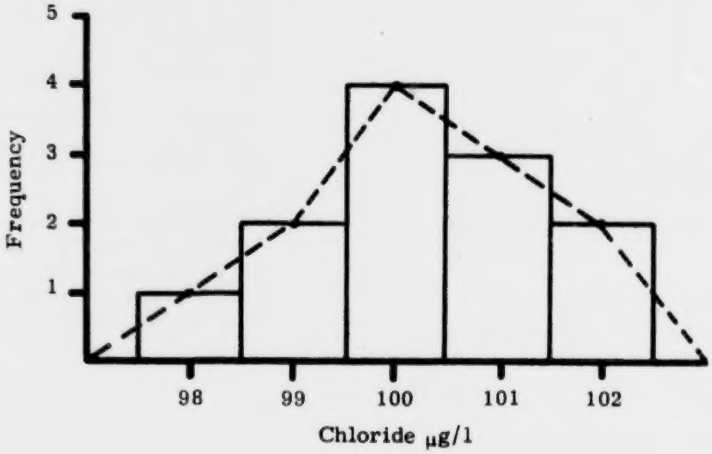
2 Frequency table - a tabular arrangement of data, ranked in ascending or descending order of magnitude, together with the corresponding frequencies

3 Frequency histogram - a set of rectangles having bases on a horizontal axis with centers at the given scores and heights equal to the corresponding frequencies (See Figure 1)

4 Frequency polygon - a line graph of frequencies plotted against scores (can be obtained by connecting midpoints of tops of rectangles in the frequency histogram) (See Figure 1)

Figure 1

Frequency Histogram & Frequency Polygon



B Application

Consider the application of the above definitions to the following set of data, obtained from twelve determinations for chloride in water.

Results (µg/l)

100	101	99
101	100	100
99	102	100
98	101	102

Table 1

Frequency Table

Chloride (µg/l)	Frequency
98	1
99	2
100	4
101	3
102	2

III MEASURES OF CENTRAL TENDENCY

A Definitions

1 Central tendency - the tendency of values to cluster about a particular value in the distribution

2 Mode - that value which occurs most frequently

3 Median - midpoint of an array of scores. If there is an odd number of observations, n, the median is $X_{\frac{n+1}{2}}$ where $X_{\frac{n+1}{2}}$ represents the $\frac{n+1}{2}$ value in the frequency distribution. If there is an even number of observations the median is $\frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2}$, the average of the middle two scores.

4 Mean - arithmetic average of all the values in the sample distribution, denoted by $\overline{X}$. The formula for calculating the sample mean is

$$\overline{X} = \frac{X_1 + X_2 + X_3 \ldots X_n}{n}$$

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

$\overline{X} = \frac{\Sigma X_i}{n}$ where there are n number of values.

B Aids in calculation of the mean

Application of the following two statements can reduce errors and amount of time spent in calculating the mean of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution is equivalent to adding or subtracting the same constant to or from the mean of the distribution. Thus the following formula:

$\overline{X}_c = \overline{X} \pm C$ where the X_i's are the values in the distribution with mean $\overline{X}$, and the $X_i \pm C$'s are the values in the distribution with mean $\overline{X}_c$.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the mean of the distribution by the same constant. Thus the following formulas:

(1) $\overline{X}_c = C\overline{X}$

or

(2) $\overline{X}_c = \frac{\overline{X}}{C}$ where the X_i's are the values in the distribution with mean $\overline{X}$,

and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with mean $\overline{X}_c$

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in Table 1.

1 Mode = 100

2 $$\text{Median} = \frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2} = \frac{X_6 + X_7}{2}$$

$$= \frac{100 + 100}{2} = 100$$

3 $$\text{Mean} = \frac{\Sigma X_i}{n}$$

$$= \frac{98 + 2\,(99) + 4\,(100) + 3\,(101) + 2\,(102)}{12}$$

$$= 100.25$$

4 Aid in Calculation

Consulting Table 1 and observing that the values are in the neighborhood of 100 we might subtract 100 from each score and obtain the following distribution:

Table 2

Frequency Table

Chloride (μg/l)	Frequency
-2	1
-1	2
0	4
1	3
2	2

Denote the mean of the distribution in Table 1 by $\overline{X}_c$. If we add 100 to each score in the distribution in Table 2, we obtain the scores in the distribution in Table 1; likewise if we add 100 to the mean, X, of the distribution in Table 2, we obtain the mean, $\overline{X}_c$, of the distribution in Table 1.

Thus $\overline{X}_c = \overline{X} + 100$

$$\overline{X}_c = \frac{\Sigma X}{n} + 100$$

$$\overline{X}_c = \frac{1(-2) + 2(-1) + 4(0) + 3(1) + 2(2)}{12} + 100$$

$$\overline{X}_c = .25 + 100 = 100.25$$

IV MEASURES OF DISPERSION

A Definitions

1 Dispersion - spread or variability of observations in a distribution

2 Range - the difference between the highest value and the lowest value

$R = \max - \min$

3 Average deviation - the sum of the deviations of the values from their mean, without regard to sign, divided by the total number of data values (n)

The formula for calculating the average deviation is:

$$d = \frac{\Sigma |X_i - \overline{X}|}{n}$$

4 Average deviation of the mean (D) - the average deviation of individual data items from the mean (d) divided by the square root of the number of data items (n)

The definition of the average deviation of the mean can be expressed by the formula:

$$D = \frac{d}{\sqrt{n}}$$

5 Variance - the sum of the squares of the deviations of the values from their mean divided by the total number of data values (n) minus 1

The definition of the variance can be expressed by the following formula:

$$s^2 = \frac{\Sigma(X_i - \overline{X})^2}{n - 1}$$

6 Standard deviation - the square root of the variance

The definition of the standard deviation can be expressed by the following formula:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n - 1}}$$

However, the formula commonly used because of its adaptability to the hand calculator is the following:

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}}$$ where there are n number of values.

7 Standard deviation of the mean (S) - the standard deviation of individual data items (s) divided by the square root of the number of data items (n)

The definition of the standard deviation of the mean can be expressed by the formula:

$$S = \frac{s}{\sqrt{n}}$$

8 Relative standard deviation - the standard deviation (s) expressed as a fraction of the mean, $\frac{s}{\overline{X}}$

The relative standard deviation is often expressed as a percent. It is then referred to as the coefficient of variation (V):

$$V = \frac{s}{\overline{X}} \times 100 = \%$$

The relative standard deviation is particularly helpful when comparing the precision of a number of determinations on a given substance at different levels of concentration.

B Aids in Calculation

Application of the following statements can reduce errors and amount of time spent in calculating the variance or standard deviation of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution doesn't affect the variance or standard deviation of the distribution.

Thus the following formulas:

(1) $s_c^2 = s^2$

(2) $s_c = s$

where the X_i's are the values in the distribution with variance s^2 and standard deviation s, and the $X_i \pm C$'s are the values in the distribution with variance s_c^2 and standard deviation s_c.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the variance of that distribution by the square of the same constant.

Thus the following formulas:

(1) $s_c^2 = C^2 s^2$

(2) $s_c^2 = \frac{s^2}{C^2}$

where the X_i's are the values in the distribution with variance s^2, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with variance s_c^2.

3 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the standard deviation of that distribution by the same constant.

Thus the following formulas:

(1) $s_c = Cs$

or

(2) $s_c = \frac{s}{C}$

where the X_i's are the values in the distribution with standard deviation s, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with standard deviation s_c.

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in II B, Table 1.

1 Range = 102 - 98 = 4

2 Average deviation - $d = \frac{\Sigma|X_i - \bar{X}|}{}$

n	X_i	$\lvert X_i - \bar{X}\rvert$	$n\lvert X_i - \bar{X}\rvert$
1	98	2.25	2.25
2	99	1.25	2.50
4	100	.25	1.00
3	101	.75	2.25
2	102	1.75	3.50
	$\bar{X} = 100.25$		11.50

$$d = \frac{\Sigma|X_i - \bar{X}|}{n} = \frac{11.50}{12} = .96$$

3 Average deviation of the mean -

$$D = \frac{d}{\sqrt{n}}$$

Using calculations from number 2,

$$D = \frac{d}{\sqrt{n}} = \frac{0.96}{\sqrt{12}} = \frac{0.96}{3.46} = 0.28$$

4 Variance - $s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n-1}$

n	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	$n(X_i - \bar{X})^2$
1	98	-2.25	5.06	5.06
2	99	-1.25	1.56	3.12
4	100	- .25	.06	.24
3	101	+ .75	.56	1.68
2	102	+1.75	3.06	6.12
				16.22

$$s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n - 1} = \frac{16.22}{11} = 1.47$$

5 Standard deviation - $s = \sqrt{\dfrac{\Sigma X_i^2 - \dfrac{(\Sigma X_i)^2}{n}}{n-1}}$

n	X_i	nX_i	X^2_i	nX^2_i
1	98	98	9604	9604
2	99	198	9801	19602
4	100	400	10000	40000
3	101	303	10201	30603
2	102	204	10404	20808
		1203		120617

$$s = \sqrt{\dfrac{120617 - \dfrac{1203^2}{12}}{11}} = \sqrt{\dfrac{120617 - 120601}{11}}$$

$$s = \sqrt{\dfrac{16}{11}} = 1.21$$

6 Aid in calculation

Recalling that adding or subtracting a constant to each score in the distribution doesn't affect the variance or the standard deviation of the distribution we can simplify the computations by first subtracting 100 from each score in the distribution, thus obtaining the frequency distribution shown in Table 2.

n	X_i-C	n(X_i-C)	$(X_i\text{-}C)^2$	$n(X_i\text{-}C)^2$
1	-2	-2	4	4
2	-1	-2	1	2
4	0	0	0	0
3	1	3	1	3
2	2	4	4	8
		3		17

$$s^2 = \dfrac{\Sigma X_i^2 - \dfrac{(\Sigma X_i)^2}{n}}{n-1}$$

$$s^2 = \dfrac{17 - \dfrac{(3)^2}{12}}{11} = \dfrac{16.25}{11} = 1.48$$

$$s = \sqrt{\dfrac{\Sigma X_i^2 - \dfrac{(\Sigma X_i)^2}{n}}{n-1}} = \sqrt{1.48} = 1.22$$

7 Standard deviation of the mean -

$$S = \dfrac{s}{\sqrt{n}}$$

Using calculations from number 6,

$$S = \dfrac{s}{\sqrt{n}} = \dfrac{1.22}{\sqrt{12}} = \dfrac{1.22}{3.46} = 0.35$$

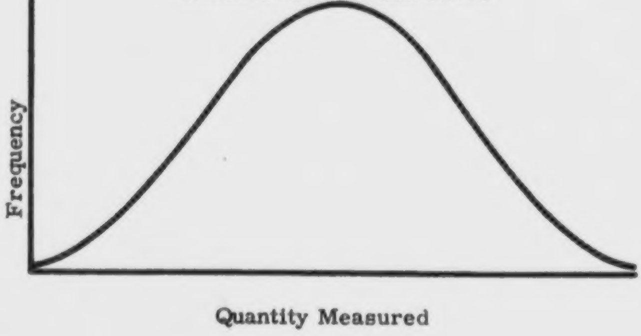
8 Relative standard deviation expressed as a percent (coefficient of variation)

$$V = \dfrac{s}{\overline{X}} \times 100$$

Using calculations from number 6 for $s = 1.22$ and from number 2 for $\overline{X} = 100.25$,

$$V = \dfrac{s}{\overline{X}} = \dfrac{1.22}{100.25} \times 100 = 1.21\%$$

Figure 2
Normal Distribution Curve



V INTRODUCTION TO NORMAL DISTRIBUTION CURVE

A Statistics deals with theoretical curves which are smoother than frequency polygons, obtained from experiments in real life. However, frequency distributions or frequency polygons of experimental data often approximate a mathematical function called the "normal" distribution curve. (See Figure 2)

As shown in Figure 3, the frequency polygon for the 12 determinations for chloride in water is a fairly good approximation of the normal curve. If, however, in the chloride determinations we had obtained 103 instead of 98 and 104 instead of 99 this distribution would not have been a good approximation of the normal curve, as is shown in Figure 4.

Figure 3

Comparison of Normal Curve and Frequency Polygon

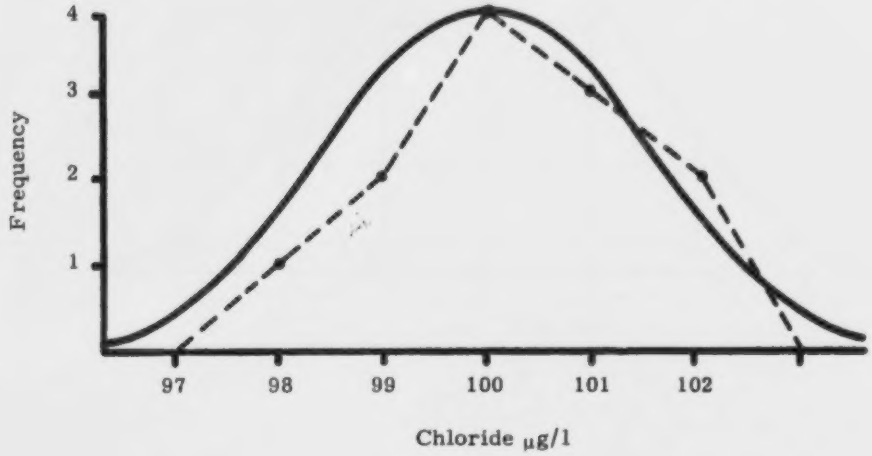
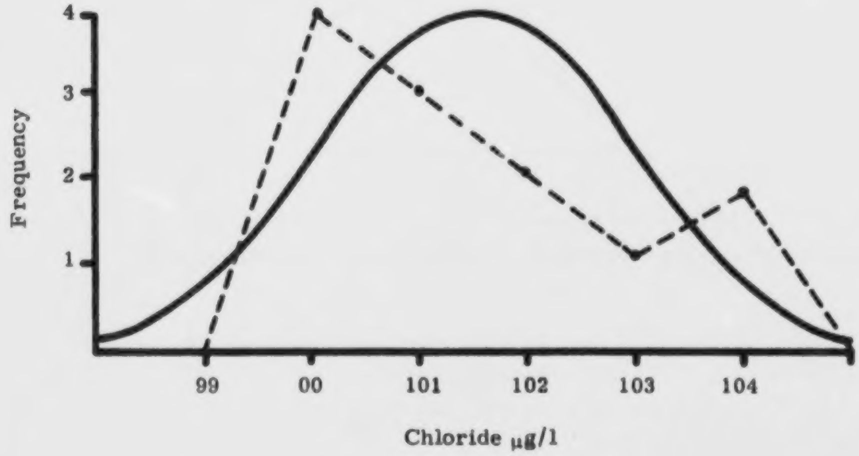


Figure 4

Comparison of Normal Curve and Frequency Polygon



B If a frequency distribution is a good approximation of the normal curve, we can use some facts about the normal curve to give us information about the frequency distribution.

Figure 5 shows the normal distribution in terms of the population mean μ, and the standard deviation of the population σ, and gives the percent of area under the curve between certain points.

Figure 5

Normal Distribution Curve

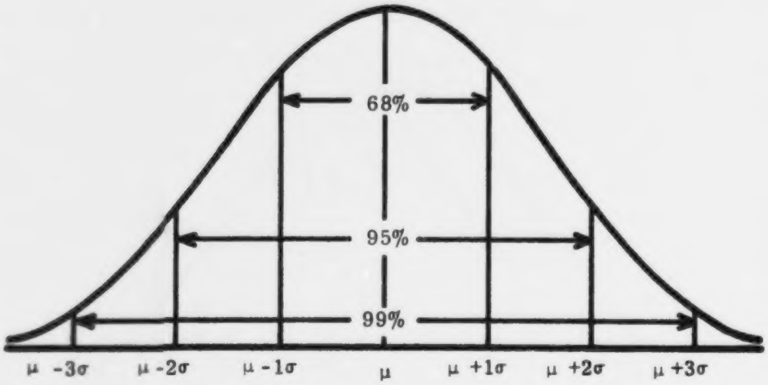
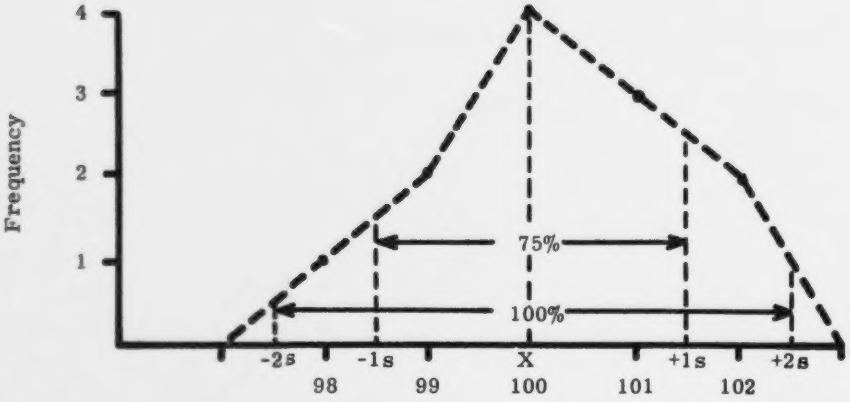


Figure 6

Frequency Distribution Polygon



Chloride μg/1

We may check the distribution of sample data to see if it is a "normal" distribution in the following manner. Substitute the value of the sample mean ($\overline{X}$) for the value of the midline and substitute the value of the sample standard deviation (s) for the limits of the value spans where we might expect certain percentages of the data items to occur. Then we can check the number of data items which actually do occur within these value spans.

Figure 6 demonstrates this application using the chloride data values from Table 1. The data values are marked on the horizontal line and the frequency of the occurrence of each value is marked on the vertical. The midline of the distribution is marked at the value of the sample mean ($\overline{X} = 100$, See III C 3). The value of the sample standard deviation ($s = 1.21$, See IV C 5) is used to mark value areas under the curve where different percentages of data values will probably occur. Thus, for the area $\overline{X} \pm 1s$, $\overline{X} - 1s = 98.79$ and $\overline{X} + 1\ s = 101.21$. Therefore, according to the normal distribution curve shown in Figure 5, we might expect about 68% of the data items to have values between 99 and 101. (The values are rounded to whole numbers since the data values are thus recorded).

Consulting Table 1, we find that 75% or 9 of the 12 data items have values in this range. This percentage is shown in Figure 6 by the frequency polygon for the data shown earlier in Figure 3.

Likewise assuming a normal distribution, we would expect 95% of the observations to lie within $\pm 2\sigma$'s from the population mean. In fact, 100% of the observations were within $\pm$ 2 s's from the sample mean.

In both cases the observed percentages are reasonably close to the expected percentages. Other tests exist for determining whether or not a frequency distribution might reasonably be assumed to approximate the normal distribution.

It would be good to become as familiar as possible with the normal distribution since an underlying normal distribution is assumed for many statistical tests of hypothesis.

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Descriptors: Graphic Methods, Quality Control, Statistical Methods, Statistics

QUALITY CONTROL

I. INTRODUCTION

The purpose of the Safe Drinking Water Act is to assure the public of an adequate supply of safe water. To achieve this, maximum levels of certain contaminants were proposed along with the prescribed methodology for analyzing for these parameters. When a laboratory performs these analyses, the laboratory should practice quality control to assure that the results being reported are true values and not in error.

Data developed from these examinations must be reliable and beyond reproach. The data can be used for making judgments on technical operations in water treatment or in legal actions involving public health hazards. For these reasons the U. S. Environmental Protection Agency in its Criteria and Procedures for the Certification of Water Supply Laboratories has set down some required and some recommended quality control procedures.

The entire section contained in the "Criteria and Procedures" document is considered as the minimum acceptable program on quality control that a laboratory can carry out and still expect reliable results. Most laboratories will want to go beyond these minimum requirements and include more quality control.

This outline will cover the required quality control procedures, then go into the recommended portions and proceed further into some ideas not in the Criteria and Procedures document. The reasons for going further are to acquaint laboratory certification personnel with sufficient information to be able to evaluate whether the laboratory has complied with the required sections and allow the Certification Officer to recommend further procedures. The topic of quality control from all aspects in a laboratory is well covered in the Handbook for Analytical Quality Control in Water and Wastewater Laboratories produced by the EPA and available from the Office of Technology Transfer. The Certification Officer should keep in mind that technical assistance to the laboratory he is evaluating is of prime importance because through this assistance he can upgrade the laboratory to produce better results.

Assistance to state Certification Officers can be obtained from the regional certification authority, the Analytical Quality Control Officer in the region, or from Environmental Monitoring and Support Laboratory in Cincinnati, Ohio.

The Quality Control section of the Criteria and Procedures document has been attached for the use of the trainee.

II. QUALITY CONTROL FOR CHEMICAL ANALYSIS

A. Mandatory Requirements

1. All quality control data must be available for inspection
2. Laboratory must analyze an unknown performance sample (when available) once per year for parameters measured. Results must be within $\pm$ 2 standard deviations of precision given in approved methods. If problems arise, appropriate technical assistance will be provided, and a follow-up performance sample should be analyzed.

3. Minimum Daily Quality Control

   a. After a standard reagent curve composed of a minimum of a reagent blank and three standards has been prepared, subsequent standard curves must be verified by use of at least a reagent blank and one standard at mid-range of the working curve. Daily checks must be within ± 10 percent of original curve.

   b. If 20 or more samples per day are analyzed, the working standard curve must be verified by running an additional standard at mid-range every 20 samples. Checks must be within ± 10 percent of original curve.

B. Guidelines

1. Current service contract on all balances.

2. Class S weights available to make periodic checks on balances.

3. Thermometer certified by the National Bureau of Standards available to check thermometers in ovens, etc.

4. Color standards or their equivalent available to verify wavelength settings on spectrophotometers.

5. Chemicals dated upon receipt of shipment and replaced as needed or when shelf life has been exceeded.

6. Criteria established for a laboratory analyzing supply samples other than its own:

   a. Laboratory should perform on a known reference sample (when available) once per quarter for the parameters measured. The measured value should be within ± 2 standard deviations of the known value, based on precision given in the approved method.

   b. At least one duplicate sample should be run every 10 samples, or with each set of samples, to verify precision of the method. Checks should be within ± 2 standard deviations.

   c. Standard deviation should be calculated and documented for all measurements being conducted.

   d. Quality control charts or a tabulation of mean and standard deviation should be used to document validity of data on a daily basis.

C. Required

1. All quality control data must be available for inspection. This statement assures the availability of the data. The person certifying the laboratory might wish to make use of these data to assure himself that the laboratory is practicing quality control and to what extent. After an amount of data have accumulated, it can serve as a record of

a continuing type of quality control rather than a sporadic, hit or miss type. At any time, should there be questions on the reliability of any data, the quality control records will be available to show the reliability of the data produced during the time period in question.

The quidelines for data reporting recommend that the records of chemical analyses should be kept by the laboratory for not less than three years. It would seem prudent that all quality control data be kept for a like period of time.

Data required would include a record of the results of the yearly performance sample, a standard curve for each method the laboratory has been certified for, the records showing a check of this curve daily or each time the analysis is carried out. If the laboratory analyzes 20 or more samples per day, records should include the value of a standard run after every 20 samples. Again, this is for a minimal program and it would be well for laboratories to adopt at least the recommended procedures listed in the Criteria and Procedures document.

2. Laboratory must perform on an unknown performance sample once per year for parameters measured.

In a minimal program this yearly check sample would be the first external indication of a problem in a laboratory to the certifying authority. The required daily quality control data would not be sent to the certifying authority. If unacceptable answers were obtained for one or more parameters, the laboratory would be asked to analyze a follow-up performance sample. If continued problems existed, the certifying authority could offer some form of technical assistance to rectify the problem. If the data is borderline or perhaps sporadic in nature, the Certifying Officer might wish to schedule his next visit at a time when the questionable analytical method is being performed.

The principal state laboratory, as well as local laboratories, will be required to analyze an unknown performance sample. This sample will be provided by the regional authority which will certify that laboratory in each state. The U. S. Environmental Protection Agency also plans to make available to states samples which can be used as performance samples for local laboratories which the state has responsibility for certifying. The performing laboratory will be given results of their analysis in terms of being within or out of the acceptable limits.

Results must be within ± 2 standard deviations of precision given in the approved methods.

The laboratory will be informed if they have or have not complied with this requirement by the authority supplying the performance sample.

3. A standard curve must be prepared and kept for each parameter the laboratory analyzes for. This curve must be prepared with a minimum of blank and three standards. The references for the analytical methods will provide the laboratory with the range of the test. Good procedure would dictate choosing the three standards to cover this entire range. A high, low and mid-range standard would be best to run. In order to assure good coverage the laboratory should be encouraged to run more than the minimal requirements as listed above. A good recommended procedure is to prepare the initial curve with a blank and eight standards covering the entire range.

   If more than one analyst will run the same test, it would be wise to have each analyst check their procedural technique by checking the standard curve.

   After the initial curve has been established, it should be verified by the use of at least a reagent blank and one standard which has concentration at mid-range of the working curve. Again, the Certification Officer should encourage more than the required minimum daily check. The recommendation for good technique recommends a blank and two standards, one high and one low concentration.

   These required daily checks of the standard reagent curve should be within ± 10% of the original concentration value. For example, if a mid-range value of 0.5 was chosen, a standard at this level analyzed as an unknown should fall between 0.55 and 0.45. If not, the analyst should check in the following order:

   a. Any variable instrument parameters

   b. Rerun check sample

   c. Prepare new standard

   d. Prepare all reagents fresh

   e. Check shelf life of chemicals

   f. Check instrument.

   If the value persists at the new value through all this, then the analyst should prepare a new standard curve.

   The laboratory Certification Officer should check to see if the daily records indicate that the curve has been checked (blank and 1 standard) and verified after each 20 samples with a standard run.

D. Guidelines

The following items are classed in the Criteria and Procedures document as recommended. However, a certain amount of importance must be attached to each item. The committee preparing the document felt strongly enough about these items to keep them in the document. Common laboratory practice would assure that these items be carried out.

1. Current Service Contract on All Balances

   The analytical balance is of great importance in a laboratory. As reagents are weighed on this piece of equipment, care must be taken to assure that it is in good working order. The laboratory Certification Officer should question the head chemist as to the existance of a service contract on the balances. Should the laboratory Certification Officer need additional information on proper care of a balance there is a section in the Handbook for Analytical Quality Control in Water and Wastewater Laboratories.

2. Class S Weights Available to Make Periodic Checks on Balances.

   This could be included as part of the routine service contract or a set purchased and shared with the bacteriological laboratory which will also have need for them. A very complete set of directions for checking the performance of a balance is contained in Part 30 of ASTM Standards.

3. NBS - Certified Thermometer Available to Check Thermometers in Ovens, etc.

   Again this item could be a shared item between chemical and bacteriological laboratories. The Certification Officer could carry this item with him and provide this service to the smaller type laboratories. Since this item is only recommended, the Certification Officer can only question if this thermometer is avaialble and used.

4. Color Standard or Their Equivalent Available to Verify Wavelength Settings on Spectrophotometers.

   The spectrophotometer like the balance is a very important piece of laboratory equipment. The Certification Officers should make themselves thoroughly aware of the proper techniques for care, use and calibration of a spectrophotometer. Again the Handbook for Analytical Quality Control is a good place to start.

   Spectrophotometers should be checked for wavelength alignment. If a particular colored solution is to be used at a closely specified wavelength, considerable loss of sensitivity can be encountered if the wavelength control is misaligned. In visual instruments, an excellent reference point is the maximum absorbance for a dilute solution of potassium permanganate, which has a dual peak at 526 mμ and 546 mμ. On inexpensive graphing instruments, which possess less resolution than the prism instruments, the permanganate peak appears at 525 to 550 mμ as a single flat-topped spike.

   Another point that should be mentioned is the care and use of spectrophotometric absorption cells. If possible, the Certification Office should observe the techniques of the laboratory in the use of the cells. Good techniques here could indicate good technique in all the colorimetric procedures.

5. Chemical Dated Upon Receipt of Shipment and Replaced as Needed or When Shelf Life is Exceeded.

   It should not be necessary to store clean glassware or chemicals on bench tops. Floor length cabinets or above bench cabinets should be available for storage. Chemicals themselves should be of analytical reagent grade to assure good quality. Dating the chemical upon receipt will give the chief chemist an indication of the amounts to order and if the chemical can still be relied on to have its initial quality.

6. Laboratories Analyzing Water Supply Samples Other Than Its Own Should Carry Out Additional Quality Control. This section covers additional recommended items for the larger laboratories.

   a. Laboratory should perform on a known reference sample (when available) once per quarter for the parameters measured.

      Since the yearly known performance sample will not indicate to the laboratory how well it is doing, other than pass or fail, a known sample will show how the laboratory compares in precision and accuracy to that given for the various methods. Analysis of the known sample will allow comparsion and show any trend of the quality control of the laboratory. These data should be available to the Certification Officer for inspection.

      This known quality control check sample should be available to the laboratory from the principal state laboratory. If not, a synthetic sample prepared by the head chemist can be used. This control can be a large sample from a natural source known to contain the constituents of concern or a synthetic sample prepared in the laboratory from chemicals of the highest purity grade. In either case, if the control is to be kept, it should be stabilized by addition of a suitable preservative. See the section on sampling for the choice of preservative.

   b. The measured value should be within ± 2 standard deviations of the known value as based upon the precision given in the approved method.

      The appropriate precision data can be found in one or the other standard references. That is

      1) Standard Methods for the Examination of Water and Wastewater, 13th Edition (1971).

      2) Manual of Methods for the Chemical Analysis of Water and Wastes, 1974 Edition.

      These data have been accumulated in Table I. If this data does not fulfill the need of the Certification Officer, he may write to the U. S. Environmental Protection Agency, EMSL, Cincinnati, Ohio 45268 and request additional information on accuracy and precision.

c. Run at least 1 duplicate sample every 10 samples or with each set of samples to verify the precision of the method. Checks should be within ± 2 standard deviations.

In order to document that reproducible results are being obtained (i.e. precision of the method), it is necessary to run duplicate samples. Although the frequency of such replicate analysis is, by nature dependent on such factors as the original precision of the method, the reliability of the instrumentation involved and the experience of the analyst, good laboratory technique is to run duplicate analysis at least ten percent of the time. The resulting data should agree within ± 2 standard deviations. These data have been collected in Table I. If the data do not agree, the system is not under control, and results are subject to question.

d. Standard deviation should be calculated and documented for all measurements being conducted.

This calculation will provide the upper and lower control limits for the test. Analysts can then determine whether or not the data produced is acceptable. This data can be calculated on seven replicate determinations for initial comparsion. However, as additional determinations are performed, they should be added to existing data and the precision data recalculated. Twenty or more runs tend to present better statistical data.

Standard deviation calculations should be determined for each analyst to carry out the analysis. However, the data should not be collected until the analyst is familiar with the procedure. The concentration used to calculate the standard deviation should be at the level expected in the sample for those laboratories doing only their own water. For laboratories doing determinations other than their own supply it would be best to have the standard deviation calculated at several concentrations. However, for a minimal effort, the concentration should be chosen at or close to the maximum contaminant level for the parameter.

In order to assure this data is collected, the standard run after each 20 samples could be at the concentration used to determine the standard deviation. This would produce a constant flow of this data for inclusion in future updates of the standard deviation calculation.

e. Quality control charts or a tabulation of mean and standard deviation should be used to document the validity of data on a daily basis.

If the upper and lower control limits of ± 2 standard deviations are calculated, the analyst will have some idea as to the acceptability of each determination as the results are obtained. When outliers are found the analyst can reschedule these for analysis to assure themselves of the result before action is taken to call for a resample of the supply.

Production of quality control charts and subsequent graphing of the charts of data obtained in the laboratory will give pictorial representation of the control of the method. Tendencies toward one or the other control limit will indicate loss of control of the method.

How to produce quality control charts and a discussion of these statistical tools is covered in the Basic Statistics outline and in the Handbook for Analytical Quality Control in Water and Wastewater Laboratories.

TABLE I

Maximum Contaminant Level µg/l	Parameters	Conc. µg/l	Standard[1] Deviation S µg/l	Relative Standard Deviation RSD%	Reference
50	Arsenic (Gaseous Hydride)	20.0	± 1.1		E.P.A. Methods - 95
		10.0		6.0	Std. Methods (14th) 146[2]
1000	Barium (Standard cond.)	40.0	43		E.P.A. Methods - 98
		1000		8.9	Std. Methods (13th) 215
		500		10.0	Std. Methods (14th) 146
10	Cadmium (Extracted)	10		72.8	Std. Methods (13th) 213
		50		43.8	Std. Methods (14th) 148
50	Chromium (Standard cond.)	74	29		E.P.A. Methods - 106
		50		26.4	Std. Methods (13th) 215
		50		26.4	Std. Methods (14th) 146
50	Lead (Extracted)	50		23.5	Std. Methods (13th)
		50		23.5	Std. Methods (14th)
2	Mercury (Flameless AA Inst.)	3.4	1.49		E.P.A. Methods - 125
		0.4		21.2	Std. Methods (14th)
10	Selenium (Gaseous Hydride)	10	1.1		E.P.A. Methods
		10		11.0	Std. Methods (14th)
50	Silver (Standard cond.)	50		17.5	Std. Methods (13th)
10,000	Nitrate (Brucine)	5000		15.4	Std. Methods (13th)
	Cadmium Red	1040	10		E.P.A. Methods - 206
		5000		9.2	Std. Methods (13th) 464
Varies with Temperature	Fluoride (SPADNS with Distil)	570		17.2	Std. Methods (13th)
		570	130		E.P.A. Methods - 59
	Electrode	750		4.8	Std. Methods (13th)
		900		2.9	Std. Methods (13th)
		750	36		E.P.A. Methods -67

[1] Where more than one concentration and Standard Deviation is given in the same reference the closest to the maximum contaminant level has been given.

[2] Although not an official reference, data included here.

III. SUMMARY

The quality control items in the Criteria and Procedures document identify a minimal effort for all types of laboratories. Since quality control is for the benefit of the laboratory in assuring valid data, it would seem wise for all laboratories to practice a good deal more quality control than set down in the Criteria Procedures document.

This section has discussed the quality control steps to be taken to assure proper analytical performance in the laboratory. However, a complete picture of quality control would include assurance to proper sampling techniques, including collection, preservation and handling; use of acceptable methods, and proper reporting of data to be considered. It must be recognized (and practiced), however, that quality control begins with collection and does not end until resulting data are reported.

LABORATORY SAFETY PRACTICES

I INTRODUCTION

A Safe Use, Handling and Storage of Chemicals

1 Chemicals in any form can be safely stored, handled, and used if their hazardous physical and chemical properties are fully understood and the necessary precautions, including the use of proper safeguards and personal protective equipment are observed.

2 The management of every unit within a manufacturing establishment must give wholehearted support to a well integrated safety policy.

B General Rules for Laboratory Safety

1 Supervisory personnel should think "safety." Their attitude toward fire and safety standard practices is reflected in the behavior of their entire staff.

2 A safety program is only as strong as the worker's will to do the correct things at the right time.

3 The fundamental weakness of most safety programs lies in too much lip service to safety rules and not enough action in putting them into practice.

4 Safety practices should be practical and enforceable.

5 Accident prevention is based on certain common standards of education, training of personnel and provision of safeguards against accidents.

II LABORATORY DESIGN AND EQUIPMENT

A Type of Construction

1 Fire-resistant or noncombustible

2 Multiple story buildings should have adequate means of exit.

3 Stairways enclosed with brick or concrete walls

4 Laboratories should have adequate exit doors to permit quick, safe escape in an emergency and to protect the occupants from fires or accidents in adjoining rooms. Each room should be checked to make sure there is no chance of a person being trapped by fire, explosions, or release of dangerous gases.

5 Laboratory rooms in which most of the work is carried out with flammable liquids or gases should be provided with explosion-venting windows.

B Arrangement of Furniture and Equipment

1 Furniture should be arranged for maximum utilization of available space and should provide working conditions that are efficient and safe.

2 Aisles between benches should be at least 4 feet wide to provide adequate room for passage of personnel and equipment.

3 Desks should be isolated from benches or adequately protected.

4 Every laboratory should have an eye-wash station and a safety shower.

C Hoods and Ventilation

1 Adequate hood facilities should be installed where work with highly toxic or highly flammable materials are used.

2 Hoods should be ventilated separately and the exhaust should be terminated at a safe distance from the building.

3 Make-up air should be supplied to rooms or to hoods to replace the quantity of air exhausted through the hoods.

4 Hood ventilation systems are best designed to have an air flow of not less than 60 linear feet per minute across the face of the hood, with all doors open and 150, if toxic materials are involved.

5 Exhaust fans should be spark-proof if exhausting flammable vapors and corrosive resistant if handling corrosive fumes.

6 Controls for all services should be located at the front of the hood and should be operable when the hood door is closed.

7 All laboratory rooms should have the air changed continuously at a rate depending on the materials being handled.

D Electrical Services

1 Electrical outlets should be placed outside of hoods to afford easy access and thus protect them from spills and corrosion by gases.

2 Noninterchangeable plugs should be provided for multiple electrical services.

3 Adequate outlets should be provided and should be of the three-pole type to provide for adequate grounding.

E Storage

1 Laboratories should provide for adequate storage space for mechanical equipment and glassware which will be used regularly.

2 Flammable solvents should not be stored in glass bottles over one liter in size. Large quantities should be stored in metal safety cans. Quantities requiring containers larger than one gallon should be stored outside the laboratory.

3 Explosion proof refrigerators should be used for the storage of highly volatile and flammable solvents.

4 Cylinders of compressed or liquified gases should not be stored in the laboratory.

F Housekeeping

1 Housekeeping plays an important role in reducing the frequency of laboratory accidents. Rooms should be kept in a neat orderly condition. Floors, shelves, and tables should be kept free from dirt and from all apparatus and chemicals not in use.

2 A cluttered laboratory is a dangerous place to work. Maintenance of a clean and orderly work space is indicative of interest, personal pride, and safety-mindedness.

3 Passageways should be kept clear to all building exits and stairways.

4 Metal containers should be provided for the disposal of broken glassware and should be properly labeled.

5 Separate approved waste disposal cans, should be provided for the disposal of waste chemicals.

6 Flammable liquids not miscible with water and corrosive materials, or compounds which are likely to give off toxic vapors should never be poured into the sink.

G Fire Protection

1 Laboratory personnel should be adequately trained regarding pertinent fire hazards associated with their work.

2 Personnel should know rules of fire prevention and methods of combating fires.

3 Fire extinguishers (CO_2 type) should be provided at convenient locations and personnel should be instructed in their use.

4 Automatic sprinkler systems are effective for the control of fires in chemical laboratories.

H Alarms

1 An approved fire alarm system should be provided.

2 Wherever a hazard of accidental release of toxic gases exists, a gas alarm system to warn occupants to evacuate the building should be provided.

3 Gas masks of oxygen or compressed air type should be located near exits and selected personnel trained to use them.

III HANDLING GLASSWARE

A Receiving, Inspection and Storage

1 Packages containing glassware should be opened and inspected for cracked or nicked pieces, pieces with flaws that may become cracked in use, and badly shaped pieces.

2 Glassware should be stored on well-lighted stockroom shelves designed and having a coping of sufficient height around the edges to prevent the pieces from falling off.

B Laboratory Practice

1 Select glassware that is designed for the type of work planned.

2 To cut glass tubing or a rod, make a straight clean cut with a cutter or file at the point where the piece is to be severed. Place a towel over the piece to protect the hands and fingers, then break away from the body.

3 Large size tubing is cut by means of a heated nichrome wire looped around the piece at the point of severance.

4 When it is necessary to insert a piece of glass tubing or a rod through a perforated rubber or cork stopper, select the correct bore so that the insertion can be made without excessive strain.

5 Use electric mantels for heating distillation apparatus, etc.

6 To remove glass splinters, use a whisk broom and a dustpan. Very small pieces can be picked up with a large piece of wet cotton.

IV GASES AND FLAMMABLE SOLVENTS

A Gas Cylinders

1 Large cylinders must be securely fastened so that they cannot be dislodged or tipped in any direction.

2 Connections, gauges, regulators or fittings used with other cylinders must not be interchanged with oxygen cylinder fittings because of the possibility of fire or explosion from a reaction between oxygen and residual oil in the fitting.

3 Return empty cylinders promptly with protective caps replaced.

B Flammable Solvents

1 Store in designated areas well ventilated.

2 Flash point of a liquid is the temperature at which it gives off vapor sufficient to form an ignitible mixture with the air near the surface of the liquid or within the vessel used.

3 Ignition temperature of a substance is the minimum temperature required to initiate or cause self-sustained combustion independently of the heating or heated element.

4 Explosive or flammable limits. For most flammable liquids, gases and solids there is a minimum concentration of vapor in air or oxygen below which propagation of flame does not occur on contact with a source of ignition. There is also a maximum proportion of vapor or gas in air above which

propagation of flame does not occur. These limit mixtures of vapor or gas with air, which if ignited will just propagate flame, are known as the "lower and higher explosive or flammable limits."

5 Explosive Range. The difference between the lower and higher explosive or flammable limits, expressed in terms of percentage of vapor or gas in air by volume is known as the "explosive range."

6 Vapor Density is the relative density of the vapor as compared with air.

7 Underwriter's Laboratories Classification is a standard classification for grading the relative hazard of the various flammable liquids. This classification is based on the following scale:

Ether Class	100
Gasoline Class	90 - 100
Alcohol (ethyl) Class	60 - 70
Kerosene Class	30 - 40
Paraffin Oil Class	10 - 20

8 Extinguishing agents

V CHEMICAL HAZARDS

A Acids and Alkalies

1 Some of the most hazardous chemicals are the "strong" or "mineral" acids such as hydrochloric, hydrofluoric, sulfuric and nitric.

2 Organic acids are less hazardous because of their comparatively low ionization potentials. However, such acids as phenol (carbolic acid), hydrocyanic and oxalic are extremely hazardous because of their toxic properties.

3 Classification of acids

B Oxidizing Materials

1 Such oxidizing agents as chlorates, peroxides, perchlorates and perchloric acid, in contact with organic matter can cause explosions and fire.

2 They are exothermic and decompose rapidly, liberating oxygen which reacts with organic compounds.

3 Typical hazardous oxidizing agents are:

Chlorine Dioxide
Sodium Chlorate
Potassium Chromate
Chromium Trioxide
Perchloric Acid

C Explosive Power

1 Many chemicals are explosive or form compounds that are explosive and should be treated accordingly.

2 A few of the more common examples of this class of hazardous materials are:

Acetylides
Silver Fulminate
Peroxides
Peracetic Acid
Nitroglycerine
Picric Acid
Chlorine and Ethylene
Sodium Metal
Calcium Carbide

D Toxicity

1 Laboratory chemicals improperly stored or handled can cause injury to personnel by virtue of their toxicity.

2 Types of exposure. There are four types of exposure to chemicals:

a Contact with the skin and eyes

b Inhalation

c Swallowing

d Injection

VI PRECAUTIONARY MEASURES

A Clothing and Personal Protective Equipment

1 Chemical laboratories should have special protective clothing and equipment readily available for emergency use and for secondary protection of personnel working with hazardous materials.

2 Equipment should be provided for adequate:

- a Eye protection
- b Body protection
- c Respiratory protection
- d Foot protection
- e Hand protection

B Bodily Injury

1 Burns, eye injuries, and poisoning are the injuries with which laboratory people must be most concerned.

2 First emphasis in the laboratory should be on preventing accidents. This means observing all recognized safe practices using necessary personal protective equipment and exercising proper control over poisonous substances at the source of exposure.

3 So that a physician can be summoned promptly, every laboratory should have posted the names, telephone numbers, and addresses of doctors to be called in an emergency requiring medical care.

REFERENCES

Guide for Safety in the Chemical Laboratory, the General Safety Committee of the Manufacturing Chemists Association, Inc., Van Nostrand, New York (1954).

This outline was prepared by Paul F. Hallbach, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Safety, Laboratory, Practices Safety, Laboratory Design Chemical Storage, Gas Cylinders, Flammable Solvents

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for

DETERMINATION OF SILVER (Ag+)

as applied in

WATER AND WASTEWATER TREATMENT FACILITIES

and in the

MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

CH.ME.ag.lab.WMP.1.11.77

WATER MONITORING PROCEDURE: Determination of Ag+

1. Analysis Objective:

   To determine the silver concentration of a water sample.

2. Brief Description of Analysis:

   The sample is digested with concentrated nitric acid and evaporated to dryness. The residue is treated with hydrochloric acid, silicates and other insoluble material are removed by filtration and the sample is analyzed for the total metal of interest by atomic absorption spectrophotometry.

3. Applicability of this Procedure:

   The method works for both potable and wastewater.

   a. Range of Concentration - The method is recommended for use in the range of 0.1 to 4.0 mg/l. The detection limit is 0.01 mg/l.
   b. Pretreatment of Sample - Digestion in acid pH to assure solubilization - See Section A.
   c. Treatment of Interferences in the Sample - None listed for these conditions.

Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, p. 146

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WATER MONITORING PROCEDURE: Determination of Ag+

Operating Procedures:

A. Sample Digestion

B. Reagent Preparation

C. Instrument Calibration

D. Instrumental Analysis

E. Calculations

WATER MONITORING PROCEDURE: Determination of Ag+

General Description of Equipment and Supplies Used in the Process

A. Capital Equipment

1. Balance, analytical - sensitivity 0.1 milligram
2. Atomic absorption spectrophotometer and recorder
3. pH meter
4. Hot plate, 110 V

B. Reusable Supplies

1. Flasks, volumetric, 100 ml, 1000 ml
2. Pipets, volumetric, 50 ml, 3 ml, 1 ml
3. Reagent bottles, glass with glass stopper
4. Anion and cation exchange resin cartridges
5. Beakers, 150 ml
6. pH paper
7. Watch glass
8. Funnel, 80 mm diameter
9. Ring stand and 3 inch ring
10. Graduated cylinders 100, 50, 10 ml

C. Consumable Supplies

1. Reagents

   Silver Nitrate (analytical reagent grade)

WATER MONITORING PROCEDURE: Determination of Ag^+

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Sample Digestion for Total Silver	1. Transfer 100 ml of sample into a clean 150 ml beaker	1a. Use a 100 ml graduated cylinder	
	2. Check the pH using pH paper	2a. The pH should be 2.0. If the sample was not acidified upon collection, add 1:1 nitric acid dropwise until the pH is adjusted to 2.0	
	3. Add 5.0 ml of 1:1 hydrochloric acid (HCl)	3a. Use a 5 ml pipet. Use a rubber bulb on the pipet	
	4. Place the beaker on a hot plate	4a. Adjust the hot plate for medium heat	
	5. Heat at 95° C for 15 min	5a. Make certain that the sample does not boil	
	6. Remove the beaker from the hot plate. Allow it to cool to room temperature		
	7. Wash down the beaker walls with distilled water	7a. Use a plastic wash bottle	
	8. Filter the sample through Whatman #42 filter paper into a clean 100 ml volumetric cylinder		
	9. Dilute the volume to 100 ml with distilled water		
B. Reagent Preparation 1. Deionized Distilled Water	1. Prepare by passing distilled water through a mixed bed of cation and anion exchange resions	1a. Use deionized distilled water for the preparation of all reagents, calibration standards and as dilution water.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)			
2. Nitric Acid Concentrated (HNO_3)	1. Commercially available reagent grade		
3. Hydrochloric Acid (HCl) 1:1	1. Prepare a 1:1 solution of reagent grade hydrochloric acid by adding 25 ml of commercially available reagent grade hydrochloric acid to 25 ml of deionized water	1a. Use a 50 ml graduate	
4. Silver Stock Standard Solution	1. Carefully weigh 1.575 grams of silver nitrate (analytical reagent grade) on an analytical balance	1a. Use a plastic weighing boat and an analytical balance	
	2. Transfer into a 1000 ml volumetric flask	2a. Use a powder funnel 2b. Use a plastic wash bottle to rinse the weighing boat and funnel into the flask	
	3. Dissolve in distilled water		
	4. Add 10 ml concentrated nitric acid	4a. Use a graduate cylinder 4b. Use caution with the acid	
	5. Dilute to mark with distilled water	5a. One ml equals 1 mg Ag+(1000 mg/liter)	
5. Fuel and Oxidant	1. Commercial grade acetylene is generally acceptable		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	2. Air may be supplied from a compressed air line, a laboratory compressor, or from a cylinder of commercial air.	2a. Caution: Air supply must be free from oil or other contaminants	
C. Instrument Calibration	1. Turn on air supply		
	2. Turn on acetylene supply		
	3. Turn on instrument and ignite flame	3a. See instruction manual for your particular instrument	
	4. Turn on power to hollow cathode lamp	4a. Select lamp for proper metal analysis	
	5. Select wave length for appropriate metal	5a. Silver (328.1 nm)	
	6. Prepare a series of standard solutions for silver as follows: Silver Transfer 1.0 ml of stock silver solution into a 100 ml volumetric flask and dilute to the mark with deionized distilled water and shake well		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Instrument Calibration (Continued)	6. Continued		
	Transfer 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the diluted standard solution into each of six 100 ml volumetric flasks respectively. Dilute to the mark with deionized distilled water and shake well. The concentration of these solutions will be 0.00, 0.02, 0.04, 0.06, 0.08, and 0.10 mg/l respectively	Use a 1 ml micro pipet graduated in 0.1 ml	
	7. Ignite flame and aspirate standard solutions into the flame		
	8. Prepare a calibration curve by plotting the concentration of the respective metals against the response for each concentration	8a. Record the response on a recorder or use the readout provided on the instrument	
D. Instrumental Analysis	1. Aspirate the unknown solution into the instrument immediately following the aspiration of the standards	1a. Flame characteristics and instrumental settings should be the same for standards and unknowns.	
	2. Record the response		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Calculations	1. Determine the concentration of the metal in the sample by substituting the observed instrumental response on the appropriate calibration curve.		

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF TOTAL CADMIUM, CHROMIUM AND LEAD BY ATOMIC ABSORPTION

as applied in

WATER TREATMENT PLANTS
and in the
MONITORING OF DISTRIBUTION SYSTEMS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.ME.lab.WMP.1.11.77

WATER MONITORING PROCEDURE: Determination of Total Cadmium, Chromium and Lead by Atomic Absorption

1. Analysis Objectives:

The user of the attached procedure will determine the cadmium, chromium and lead content of a drinking water sample, including sample preparation and atomic absorption.

2. Brief Description of Analysis:

The sample is treated with acid and heat to assure complete solubilization of the metals. Then the trivalent chromium is oxidized to the hexavalent form and chelated with ammonium pyrollidine dithiocarbomate (APDC). The chelated metals are then extracted with methyl isobutyl ketone and the organic metal containing solvent aspirated into an atomic absorption spectrophotometer.

3. Applicability of the Procedure:

a. Range of concentration:

This procedure should be carried out if the concentrations in the sample are below:

0.02 mg/l for cadmium
0.05 mg/l for chromium
0.10 mg/l for lead

b. Pretreatment of samples:

This is covered in the procedure. Generally it is to lower the pH of the sample to below pH 2 with nitric acid.

c. Treatment of interferences in samples:

The method contains steps to remove interferences.

Source of the Procedure: Standard Methods for the Examination of Water and Wastewater, 13th edition, pp. 210-215.

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WATER MONITORING PROCEDURE: Determination of Total Cadmium, Chromium and Lead by Atomic Absorption

A. Glass Ware Preparation

B. Sample Preservation and Handling

C. Reagent Preparation

D. Instrument Set-up

E. Preparation of Standard Dilutions

F. Solubilization of Sample

G. Oxidation of the Sample

H. Extraction Procedure

I. Instrument Calibration

J. Sample Analysis

K. Calculation

WATER MONITORING PROCEDURE: Determination of Total Cadmium, Chromium and Lead by Atomic Absorption

Equipment and Supply Requirements

A. Capital Equipment:

1. Atomic absorption spectrophotometer: Any commercial atomic absorption instrument having an energy source, an atomizer burner system, a monochromator, and a detector is suitable
2. Balance, analytical with a 0.1 milligram sensitivity
3. Hollow cathode lamps - cadmium, chromium, lead
4. Hot plate, capable of holding at least ten 250 ml beakers
5. pH meter (optional), to adjust pH for test
6. Pressure regulator valves:
   a. Two stage regulator designed to deliver acetylene with an inlet CGA 510 connector
   b. Two stage regulator designed to deliver air with an inlet CGA 1340 connector
7. Recorder: One compatible with the electronics of the atomic absorption instrument is acceptable
8. Still - borosilicate glass distillation apparatus or another source of good distilled water
9. Stop watch

B. Reusable Supplies:

1. Beakers, 3 - 250 ml size, 3 (sample, spike, duplicate, standards)
2. Three dropper bottles - brown glass, 100 ml size
3. Four reagent bottles - clear glass, 1000 ml capacity
4. Cylinders - graduated
   1 1000 ml
   1 500 ml
   1 250 ml
   1 10 ml
   1 10 ml glass stoppered, wide base (sample, spike, duplicate, standard)
5. Flask, volumetric, glass stoppered
   5 1000 ml
   2 200 ml
   3 100 ml
6. Funnel, very small to filter 365 ml, glass
   1 (sample, spike, duplicate, standard)
7. Funnel, separatory, glass stoppered, teflon stopcock, 250 ml
   1 (sample, spike, duplicate, standard)
8. Pipets, graduated, mohr type
   1 5 ml
   2 10 ml
   Volumetric type
   4 1 ml
   2 2 ml
   1 3 ml
   4 5 ml
   5 10 ml
   1 50 ml
9. Instrument - manufacturer's operation manual on the atomic absorption

WATER MONITORING PROCEDURE: Determination of Total Cadmium, Chromium and Lead by Atomic Absorption

Equipment and Supply Requirements (Cont'd.)

10. Safety glasses
11. Separatory funnel rack
12. Wash bottle, plastic, squeeze type
13. Watch glasses, 1 (sample, spike, duplicate), 3.5 inches in diameter

C. Consumable:

1. Deionizing column - mixed bed type
2. Gases
   Fuel, acetylene (E_2H_2) - for use with the atomic absorption instrument, purified grade, 380 cf, CGA 510
   Oxidant, air - for use with the atomic absorption instrument, dry grade, 2200 cf, CGA size 1340
3. Filter paper - Whatman #42
4. Plastic weighing boats - about 12
5. Labels
6. Marking pencil
7. Reagents
   Ammonium pyrrolidine dithiocarbamate
   Ammonium hydroxide
   Nitric acid
   Hydrochloric acid
   Methyl isobutyl ketone
   Cadmium sulfate
   Chromium trioxide
   Lead nitrate
   Potassium permanganate

   pH adjustment - only if the indicator is to be used
   95% ethyl alcohol
   Bromphenol blue

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Glassware Preparation	1. Wash in tap water with detergent and brush.	1a. Do not use chromic acid to wash or if it is used, rinse with tap water until all acid has been removed. 1b. This procedure should also be used for sample containers.	
	2. Rinse well with tap water.	2a. Remove all detergent.	
	3. Rinse with 1:1 nitric acid.	3a. Preparation can be found in the reagent preparation section.	
	4. Rinse with deionized distilled water.	4a. Preparation can be found in the reagent preparation section.	
	5. Reserve all glassware used in metal analysis for that purpose only.	5a. Contamination from other reagents is less likely this way.	
B. Sample Preservation and Handling	1. Collect at least a 1 liter sample.	1a. A quart sample container may be used. 1b. The sample container should have been cleaned using the procedure above.	
	2. Add 2.0 ml of concentrated nitric acid per liter of sample.	2a. The pH must be less than 2. 2b. The nitric acid should be checked for metals content before use. 2c. More acid may be necessary for samples with higher total dissolved solids.	
	3. The sample may be kept for 6 months before analysis.	3a. Good practice would dictate analysis of a sample as soon after collection as possible.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation			
1. Deionized Distilled Water	1. Prepare approximately ten (10) liters of deionized distilled water.	1a. Prepare by passing distilled water through a mixed bed of cation and anion exchange resins. 1b. Use deionized distilled water for preparation of all standards, reagents and dilutions and also for the washing of equipment.	
2. Nitric Acid Concentration	1. No preparation necessary.	1a. Check for metal impurities; if present, distill in all borosilicate glass still or find an uncontaminated source. 1b. Smaller amounts may be prepared. Use equal amounts of acid and water. 1c. Prepare in a well ventilated area.	
3. Nitric Acid (1:1)	1. Add 500 ml of water to a 1000 ml graduated cylinder.	1a. Deionized distilled water.	
	2. Add 500 ml of concentrated nitric acid to the same graduated cylinder.	2a. Caution: Do not reverse this order of addition. 2b. Use safety glasses. 2c. Heat may be generated.	
	3. Allow to cool.		
	4. Transfer into screw capped bottle for storage.		
	5. Label bottle 1:1 nitric acid.		
4. Hydrochloric Acid Concentration	1. No preparation necessary.	1a. Check for metal impurities; if present, distill in all borosilicate glass still or find uncontaminated source.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)	1. Add 500 ml of water to a 1000 ml graduated cylinder.	1a. Deionized distilled water. 1b. Smaller amounts may be prepared. Use equal amounts of water and acid. 1c. Prepare in well ventilated area.	
	2. Add 500 ml of concentrated hydrochloric acid to the same graduated cylinder.	2a. Caution: do not reverse this order of addition. 2b. Use safety glasses. 2c. Heat may be generated.	
	3. Allow to cool.		
	4. Transfer into screw capped bottle for storage.		
	5. Label bottle 1:1 hydrochloric acid.		
5. Hydrochloric Acid (0.60 N)	1. Add about 50 ml water to a 100 ml volumetric flask.	1a. Use a reagent grade analyzed acid to avoid contamination. 1b. Caution: protective equipment should be used.	
	2. Pipet 5 ml concentrated HCl to the flask.	2a. Use a 5 ml volumetric pipet.	
	3. Cool and dilute to 100 ml with water.	3a. Store in dropper bottle (about 100 ml vol.) before use.	
6. Ammonium Hydroxide (NH_4OH) Concentrated	1. Pour the concentrated NH_4OH into a glass dropper bottle.	1a. Use a hood to prevent inhalation of fumes. Avoid contact with skin. Wear protective equipment. 1b. Only some drops of this are needed for the pH adjustment of acidified samples. 1c. A brown glass dropper bottle conserves the stability of this reagent.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
7. Ammonium Hydroxide (NH_4OH) 2N	1. Dilute 3 ml of concentrated NH_4OH to 100 ml with water.	1a. This reagent should be prepared in the hood to prevent inhalation of fumes. Avoid contact with skin. Wear protective equipment. 1b. Only several drops are needed per sample. 1c. Use a brown dropper bottle to store.	
8. Bromphenol Blue Indicator	1. Dissolve 0.1 g of the solid in 100 ml of 50% ethyl alcohol.	1a. A platform balance can be used for weighing. 1b. In order to prepare this solution 95% ethyl alcohol should be diluted in half (i.e., 50 ml alcohol to 50 ml water). 1c. This solution is stable indefinitely so long as it is kept tightly stoppered to prevent evaporation. 1d. This solution is not necessary if a pH meter is used.	
9. Ammonium Pyrrolidine dithiocarbamate (4% w:v)	1. Weigh out 4.0 g of ammonium pyrrolidine dithiocarbamate (APDC).	1a. Use a trip balance and a plastic weighing boat.	
	2. Add about 50 ml water to a 100 ml volumetric flask.	2a. Estimate the amount.	
	3. Transfer the APDC to the flask.	3a. Wash the weighing boat and add washings to the flask.	
	4. Dilute to the mark with water.		
	5. Mix and label.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
10. Methyl Isobutyl Ketone (HIBK)	1. No preparation necessary.	1a. Check contamination by aspiration into AA at the wavelengths of Cd_1Cr_1Pb.	
		1b. If contaminated, wash five times with 100 ml of a solution prepared by diluting 1.0 ml of concentrated hydrochloric acid to 100 ml with water; then rinsing once with deionized distilled water.	
11. Stock Cadmium Solution	1. Weigh out 2.282 grams of cadmium sulfate ($3CdSO_4 \cdot 8H_2O$).	1a. Use an analytical balance and a plastic weighing boat.	
	2. Add about 500 ml of water to a 1000 ml volumetric flask.		
	3. Transfer the cadmium sulfate to the volumetric flask.	3a. Use a wash bottle and rinse the weighing boat with water three times adding each wash to the flask.	
	4. Add 2.0 ml of concentrated nitric acid.	4a. Use a 5 ml graduated pipet.	
	5. Dilute to the mark.	5a. The solution contains 1000 mg Cd/liter (1 ml = 1 mg Cd).	
	6. Mix thoroughly.		
12. Stock Chromium Solution	1. Weigh out 1.923 grams of chromium trioxide (CrO_3).	1a. Use an analytical balance and a plastic weighing boat.	
	2. Add about 500 ml of water to a 1000 ml volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)	3. Transfer the chromium trioxide to the volumetric flask.	3a. Use a wash bottle and rinse the weighing boat with water three times adding each wash to the flask.	
	4. Add 2.0 ml of concentrated nitric acid.	4a. Use a 5 ml graduated pipet.	
	5. Dilute to the mark.	5a. The solution contains 1000 mg Cr/liter (1 ml = 1 mg Cr).	
	6. Mix thoroughly.		
13. Stock Lead Solution	1. Weigh out 1.599 grams of lead nitrate ($Pb(NO_3)_2$).	1a. Use an analytical balance and a plastic weighing boat.	
	2. Add about 500 ml of water to a 1000 ml volumetric flask.		
	3. Transfer the lead nitrate to the volumetric flask.	3a. Use a wash bottle and rinse the weighing boat with water three times adding each wash to the flask.	
	4. Add 2.0 ml of concentrated nitric acid.	4a. Use a 5 ml graduated pipet.	
	5. Dilute to the mark.	5a. With water. 5b. The solution contains 1000 mg Pb/liter (1 ml = 1 mg Pb).	
	6. Mix thoroughly.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
14. Potassium Permanganate Solution ($KMnO_4$) 0.1 N	1. Weigh out 3.16 grams of potassium permanganate.	1a. Use a trip balance and a plastic weighing boat.	
	2. Add about 500 ml of water to a 1000 ml volumetric flask.		
	3. Transfer the potassium permanganate to the flask.	3a. Wash the weighing boat with water and add the washings to the flask.	
	4. Dilute to the mark.		
	5. Mix thoroughly.		
D. Instrument Set-up			
1. Pre Warm-up	1. Prepare the instrument for initial operation.	1a. Reference is made to the manufacturer's manual of operation. 1b. Check power requirements and availability. 1c. Provide adequate ventilation, including a vent over instrument burner. 1d. Provide adequate space for instrument and work area. 1e. Provide drain facility for the instrument.	
2. Lamp Installation	1. Install appropriate hollow cathode lamp.	1a. A hollow cathode lamp for lead, chromium and cadmium must be available. 1b. If the instrument is a single beam type, some method of warm-up for the hollow cathode lamps should be available. 1c. Do not exceed the maximum current rating for the lamps as this can seriously affect its life and stability.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Set-up (Continued)		1d. Refer to the instrument manufacturers manual for proper installation procedure.	
	2. Align the lamp for maximum intensity.	2a. Check instrument manual for proper procedure.	
	3. Set appropriate wavelength.	3a. Pb, 283.3 nm; Cd, 228.8 nm; Cr, 357.9 nm.	
3. Burner Optimization	1. Install the burner head.	1a. The usual burner head for direct aspiration is the three slot Boling head. For aspiration of organic solvents a conventional head with a single slot 7.6 cm (3 inches) is used.	
	2. Attach the necessary gasses to the instrument.	2a. For this procedure, acetylene and air are used. Use purified grades of the gasses. 2b. Attach a pressure regulator to the tanks. Use a CGA fitting of 510 for the acetylene and a 590 or 1340 for the air. 2c. Connect cylinders through the regulator to the inlet part of the instrument with plastic pressure tubing. 2d. All cylinders should be securely fastened to prevent them from tipping over.	V.D.3.2a (p. 29)
	3. Align the burner to obtain optimum absorption.	3a. The analysis of lead is exceptionally sensitive to turbulence and absorption bands in the flame. Therefore, some care should be taken to position the light beam in the most stable, center, portion of the flame. To do this, first adjust the burner to maximize the absorbance reading with a lead standard. Then aspirate a water blank and make minute adjustments in the burner alignment to minimize the signal.	
4. Check Aspiration Rate	1. Optimize the aspiration rate.	1a. Aspirate a standard into the burner and adjust the aspiration rate until optimum absorbance is obtained.	

WATER MONITORING PROCEDURE: Determination of Total Cadmium, Chromium and Lead by Atomic Absorption

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Preparation of Standard Dilutions Primary Mixture	1. Add about 500 ml of water to a 1000 ml volumetric flask.	1a. Estimate the amount.	
	2. Add 2.0 ml of concentrated nitric acid.	2a. Use a 5 ml graduated pipet. 2b. Use safety glasses.	
	3. Pipet 10 ml of each stock solution into this volumetric flask.	3a. Use three 10 ml volumetric pipets.	
	4. Dilute to the mark with water.	4a. This solution contains 10 mg Cd_1Cr_1Pb/liter.	
	5. Mix thoroughly and label.		
Intermediate Mixture	1. Add about 50 ml of water to a 100 ml volumetric flask.	1a. Estimate the amount.	
	2. Add 2.0 ml of concentrated nitric acid.	2a. Use a 5 ml graduated pipet.	
	3. Pipet 10 ml of the primary mixture into the flask.	3a. Use a 10 ml volumetric pipet.	
	4. Dilute to the mark with water.	4a. This solution contains 1 mg Cd_1Cr_1Pb/liter.	
	5. Mix thoroughly and label.		
F. Dilution Series	1. Prepare a blank and a series of standard dilutions of 200 ml volumes.	1a. Using the Intermediate Mixture, prepare the blank and standards using volumetric pipets (1, 2, 5, 10, 50 ml) for measuring the intermediate mixture and a graduated cylinder for the water.	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>E. Preparation of Standard Dilutions (Continued)</td><td></td><td>1b. These should be prepared in a series of six 250 ml separatory funnels.
1c. Prepare as follows, using columns A and B.
<table>
<tr><th>A</th><th>B</th><th>C</th><th>D</th><th>E</th></tr>
<tr><th>ml's of Int. Mix</th><th>ml's of Water</th><th>Conc. (mg/l) in 200 ml</th><th>Conc. (mg/l) in Final 10 ml</th><th>Inst. Reading</th></tr>
<tr><td>0.0</td><td>200</td><td>0.000</td><td>0.00</td><td></td></tr>
<tr><td>1.0</td><td>199</td><td>0.005</td><td>0.10</td><td></td></tr>
<tr><td>2.0</td><td>198</td><td>0.010</td><td>0.20</td><td></td></tr>
<tr><td>5.0</td><td>195</td><td>0.025</td><td>0.50</td><td></td></tr>
<tr><td>10.0</td><td>190</td><td>0.050</td><td>1.00</td><td></td></tr>
<tr><td>50.0</td><td>150</td><td>0.100</td><td>2.00</td><td></td></tr>
</table>
1d. Make sure the stopcock on the separatory funnel is closed before any addition.
1e. These dilutions should be prepared fresh for each analysis.
1f. These will be used to prepare a standard curve.</td><td>VII.E.1.1b (p. 30)</td></tr>
<tr><td></td><td>2. Carry these standards through the extraction procedure.</td><td>2a. Once the standard has been determined, it need not be redone each time the analysis is carried out. However, it should be verified by running a blank and a standard at the MCL.
2b. Each time a sample is run there should be analyzed with it a:
- reagent blank
- Standard at the MCL
- Duplicate of each sample
- Spike of each sample</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Preparation of Standard Dilutions (Continued)			
Standard Mixture	1. Add about 50 ml water to a 200 ml volumetric flask.		
	2. Add 2 ml of concentrated nitric acid.		
	3. Pipet 10.0 ml of the intermediate mixture to the flask.	3a. Use a 10 ml volumetric pipet.	
	4. Dilute to the mark with water.	4a. This solution contains 0.05 mg Cd_1Cr_1Pb/liter.	
	5. Mix thoroughly.	5a. Use 200 ml of this as a standard to be run with every sample(s) to check the standard curve.	
Spiking Mix	1. Add 1.0 ml of the primary mixture to a 200 ml volumetric flask.	1a. This 1 ml in 200 ml will add 0.05 mg/l of the metals to the sample. 1b. Use a 1.0 ml volumetric pipet.	
	2. Dilute to the mark with sample.	2a. Use 200 ml of this spiked sample to run with every sample(s) to verify concentrations.	
F. Solubilization of Sample	1. Acidify the sample with 1:1 nitric acid to a pH of 2 at the time of sample collection.	1a. For most samples, 3 ml of 1:1 HNO_3 per liter of sample lowers the pH sufficiently. 1b. Highly buffered samples require more acid. If in doubt, check the pH.	

WATER MONITORING PROCEDURE: Determination of Total Cadmium, Chromium and Lead by Atomic Absorption

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Solubilization of Sample (Continued)	2. Transfer 200 ml of well mixed sample to a 250 ml beaker.	2a. 200 ml is the usual sample volume for concentrations containing less than 100 µg/liter. 2b. Choose a volume appropriate to the expected level of metals.	
	3. Add 3 ml of concentrated nitric acid to the sample.	3a. Use a 10 ml graduated pipet.	
	4. Place the beaker on a hot plate and cautiously evaporate to dryness.	4a. The sample should not boil during this time.	
	5. Cool the beaker and add another 3 ml portion of the concentrated nitric acid.	5a. Use a 10 ml graduated pipet.	
	6. Cover the beaker with a watch glass and return to the hot plate.		
	7. Increase the temperature of the hot plate so that a gentle reflux action occurs.	7a. This will be indicated by droplets forming on the underside of the watch glass.	
	8. Continue heating until a light colored residue forms.	8a. This will indicate that the digestion is complete. 8b. Additional acid may be required to complete this digestion.	
	9. Add sufficient 1:1 hydrochloric acid (HCl) solution and again warm the beaker to dissolve the residue.	9a. Use a 1 ml graduated pipet. 9b. Use the watch glass again to cover the beaker.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Solubilization of Sample (Continued)	10. Wash down the beaker walls and watch glass with distilled water.		
	11. Filter the sample into a 250 ml graduated beaker.	11a. Use a filter paper such as Whatman #42. 11b. To remove silicates and other insoluble material that could clog the atomizer of the atomic absorption instrument.	
	12. Adjust the volume to 200 ml (or the starting volume size).	12a. The sample is now ready for extraction. 12b. The graduations on the beaker can be used to measure the volume. 12c. Results shall be reported as "total."	VII.F.12 (p. 30)
	13. The sample is now ready for the Extraction Procedure (G).		
G. Oxidation of the Sample and Standards	1. Transfer 200 ml of well mixed sample or standard into a 400 ml beaker.	1a. The extraction procedure (Section H) will extract only hexavalent chromium. To determine total chromium oxidize the sample and standards.	
	2. Bring to boil on a hot plate.		
	3. Add 0.1 N potassium permanganate dropwise.	3a. A pink color must persist during the boiling period. 3b. If the color fades, add more drops of potassium permanganate.	
	4. Boil for 10 minutes.		
	5. Cool and extract (Section H)		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Extraction Procedure for Samples and Standards	1. Place a 250 ml separatory funnel into the ring on the stand.	1a. Check the stop-cock on the separatory funnel to assure that it is closed. NOTE: If a pH meter with two electrodes is to be used to adjust the pH in the next steps, do those steps before adding sample to the separatory funnel. If using a single combination electrode, this can be done in the separatory funnel.	
	2. Add 200 ml of sample to the separatory funnel.	2a. If you have a solubilized sample from Procedure E, pour the 200 ml from the beaker into the funnel, and rinse the beaker into the funnel. 2b. In other cases, use a 500 ml graduated cylinder to measure the necessary volume. Add rinse. 2c. Record sample volume used for analysis. 2d. If use of 200 ml of sample gives results too high to be on scale, a dilution can be made. However, the dilution should be made to provide a 200 ml final volume. 2e. If use of a 200 ml sample gives too low results, more than one sample aliquot can be treated and the extracts combined.	
I. pH Adjustment	1. Add 2 drops bromphenol blue indicator to the sample(s) and to each standard.	1a. Should a pH meter be used to adjust the pH, omit steps 1 through 4 and begin at step 5. 1b. Apply each step to each sample and standard.	
	2. Mix well.		
	3. Add ammonium hydroxide dropwise until a very pale blue color persists.	3a. Use concentrated NH_4OH for acidified samples and standards made with 10 or more ml of acidified working lead standard. Use 2 N NH_4OH for more dilute standards. 3b. The reagents should be in glass dripper bottles for this addition. Use a hood.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Extraction Procedure for Samples and Standards	4. Add 0.6 N hydrochloric acid (Reagent 4) dropwise until the blue color just disappears.	4a. Use a glass dropper bottle for this addition. The bottle should be labeled HCl-.6N. 4b. Pale yellow color may appear.	
	5. Add 2.0 ml 0.6 N hydrochloric acid (Reagent 4). Stopper and shake.	5a. Use a 2.0 ml volumetric pipet. 5b. If a pH meter is used, add acid until the pH is 2.3. Then, the next procedure will bring the pH to the optimum of 2.8.	
5. Chelation and Extraction	1. Add 1.0 ammonium pyrrolidine dithiocarbamate (APDC).	1a. Use a 1.0 ml volumetric pipet. 1b. This reagent should be made fresh each time the analysis is carried out.	
	2. Add 5.0 ml methyl isobutyl ketone (MIBK).	2a. Use a 5.0 ml volumetric pipet. 2b. If a precipitate forms in the solvent phase during the first extraction, add an additional 5.0 ml MIBK.	
	3. Shake vigorously for 3 minutes.	3a. If an emulsion forms at the interface of the two layers, centrifuge for 10 minutes. 3b. Caution: Use proper technique with the separatory funnel. The funnel contains volatile solvents and pressure is formed.	
	4. Allow the layers to separate.	4a. Enough time should be allowed for complete separation of the two phases.	
	5. Drain off the aqueous layer into a beaker.	5a. Use a 250 ml beaker. 5b. Make sure none of the aqueous layer remains in the stem of the funnel. 5c. The bottom phase in the water, the top in the MIBK.	
	6. Drain off the MIBK into a 10 ml glass stoppered graduated cylinder.	6a. If an additional 5.0 ml of MIBK was used in step 2b, use a larger glass stoppered graduated cylinder here.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Extraction Procedure for Samples and Standards (Continued)	7. Close the stopcock.		
	8. Pour the aqueous layer into the separatory funnel.		
	9. Rinse the beaker with 5.0 ml of MIBK.	9a. The same volumetric pipet can be used for all additions of HIBK to all samples and standards provided caution is used to prevent contamination. 9b. If an additional 5.0 ml of MIBK was added in step 2b, use a 10 ml volume here. 9c. Since the extra solvent reduces the concentration factor and sensitivity of the method, do not apply unless necessary.	
	10. Pour the MIBK from the beaker.		
	11. Shake vigorously for 3 minutes.		
	12. Allow layers to separate.		
	13. Drain off aqueous layer and discard.	13a. Make sure none of the aqueous layer is left in the stem of the funnel.	
	14. Add the second organic extract to the first in the graduated cylinder.		
	15. Bring to 10 ml volume with MIBK.		
	16. Mix by inversion.		

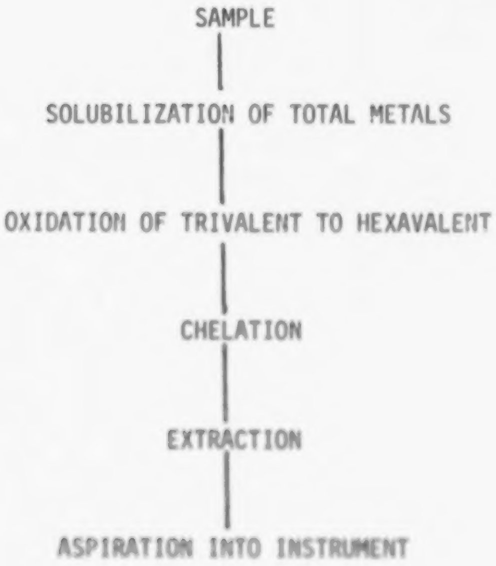
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Instrument Calibration	1. Turn on power to instrument and lamps.	1a. If the power has been turned off.	
	2. Check and adjust to optimum settings all instrumental operational parameters.	2a. Set wavelength to one of the settings given earlier. 2b. Install proper lamp. 2c. Adjust current to the lamp as listed by manufacturer. 2d. Set slit width. 2e. Ignite flame. 2f. Adjust fuel and oxidant flows. 2g. Adjust zero on recorder. 2h. See operational manual for directions.	
	3. Aspirate the blank.	3a. After extraction. 3b. When working with organic solvents, reduce the fuel-to-air ratio because the burning of the organic solvent contributes to the fuel supply, and, in addition to lifting the flame off the burner, start with the settings recommended by the manufacturer for the analysis and gradually reduce the fuel flow (while the solvent is being aspirated) until the flame is similar to the flame before aspiration of the organic solvent. In the case of air-acetylene, produce as blue, rather than white, a flame as possible.	
	4. After the standard series has been prepared (Section E), oxidized (Section G), and Extracted (Section H) aspirate, the lowest and progress toward the highest into the atomic absorption which has been set up (Section D).	4a. If the instrument is to be adjusted to read directly in concentration it may be necessary to start with the highest standard to set the slope of the absorbance. The instrument manufacturers manual will describe the procedure.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Instrument Calibration (Continued)	5. Measure and record the peak height, in millimeters, obtained on the records.	5a. The various instrument operational settings should be recorded for the record. 5b. Repeat the aspiration of the standards and blank a sufficient number of times to secure a reliable average reading for each.	
	6. Check all results before proceeding.		
	7. Plot the standard curve.	7a. Plot a curve for each metal. 7b. Plot on linear graph paper. 7c. Plot the peak height, in millimeters against the concentration, in mg metal per liter, before extraction. 7d. Check the standard curves by running at least a blank and one standard at or near the MCL. This check should be done with each sample or set of samples. The check should be within ± 10% of the original value. If not, a new standard curve should be prepared.	
D. Sample Analysis	1. Measure out 200 ml of sample.		
	2. Solubilize sample.	2a. To determine total metals. 2b. Section F.	
	3. Oxidize the sample.	3a. To convert trivalent chromium to hexavalent chromium. 3b. Trivalent chromium will not be extracted by the APDC. 3c. Section G.	
	4. Extract the sample.	4a. And all standards. 4b. Extract the metals into an organic layer and concentrate. 4c. Section H.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Sample Analysis (Continued)	5. Aspirate the organic (MIBK) solvent into the instrument.	5a. Sections I and J.	
	6. Calculate the concentration of each metal	6a. Section K.	
K. Calculations	1. Read the metal concentration in mg/liter from the appropriate calibration curve according to I.7.		

WATER MONITORING PROCEDURE: Determination of Total Cadmium, Chromium and Lead by Atomic Absorption

FLOW CHART


SAMPLE
SOLUBILIZATION OF TOTAL METALS
OXIDATION OF TRIVALENT TO HEXAVALENT
CHELATION
EXTRACTION
ASPIRATION INTO INSTRUMENT

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked.
These standardized headings are used through this series of procedures.

INTRODUCTION — Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	The National Interim Primary Drinking Water Regulations listed ten inorganic parameters which are required to be analyzed for by a public water supplier. Along with this list, a level was set for the amount of that parameter permitted to be in the water. This was the "Maximum Contaminant Level or MCL." The MCL's for the metals included in this procedure are: Cadmium - 0.010 mg per liter Chromium - 0.050 mg per liter Lead - 0.050 mg per liter	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.3.2a.	As acetylene (CHCH) is packed dissolved in acetone (CH_3COCH_3), cylinders should be stored only in an upright position. The acetone content of the gas typically depends on the cylinder temperature and pressure. Avoid introducing acetone into the instrument. Should this occur, the normal flame obtained will have a slight pink tinge and yield an abnormally high background signal. To reduce acetone carry-over, it is desirable to allow acetylene cylinders to stand undisturbed for at least twenty-four (24) hours before use. Replace the cylinder when the cylinder reaches 50 psig.	Instrumentation Laboratory Incorporated. Instrumentation Handbook 113 Hartwell Ave. Lexington, MA 02173

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

F.12

If the volume of sample is reduced sufficiently, i.e., to about 10 or 20 ml's, it might be possible to aspirate the sample directly into the atomic absorption instrument at this point for good quality drinking waters. However, high dissolved solids and sulfates may cause severe interference. The results obtained by this short-cut should be checked thoroughly before reporting.

The total solids would cause light scattering effects producing high results. The sample absorbance should be checked at a non-absorbing wavelength. If absorbance is still obtained at this wavelength, then the scattering effect is contributing to the sample value and the extraction procedure must be carried out.

Sulfates tend to suppress the absorbance of energy by lead causing low readings. With a standard concentration at the MCL accompanying all samples, this interference could be detected.

When a daily check of the standard curve falls outside the 10% limit of the procedure, reagents prepared since the last check and any recently purchased chemicals should be checked before doing a new curve.

If possible, some old chemical should be retained when a new batch is purchased. Consequently, the new may be checked against the old, should doubt arise in its purity.

F.1

The "E" Section must be carried out to produce a result for the determination of the total metal. If Section "E" is not carried out, the sample should be filtered through a 0.045 micron filter, extracted and the value reported as dissolved metal.

E.1.1b

In an effort to reduce the number of separatory funnels necessary for this determination, it is possible to prepare the blank and standards in 200 ml volumetric flasks. Then allow them to remain in the flasks while the sample or samples are carried through steps solubilization and extraction. The standards could then be extracted.

Then all, standards, blank, and samples, could be carried through the remaining steps, and brought together at step G.5.2.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF MERCURY USING THE FLAMELESS ATOMIC ABSORPTION (COLD VAPOR) TECHNIQUE

as applied in

WATER AND WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.ME.hg.lab.WMP.1.11.77

WATER MONITORING PROCEDURE: Determination of Mercury Using the Flameless Atomic Absorption (Cold Vapor) Technique

1. Analysis Objectives:

The learner will use the attached WMP to prepare a sample for analysis including reagent and sample preparation. A description of the instrumentation will be presented. However, the learner should consult the manufacturer's direction for operation of any equipment.

2. Brief Description of Analysis:

This procedure is a three step procedure which 1) chemically vaporizes the sample, 2) introduces the mercury and 3) determines the mercury by flameless atomic absorption techniques.

3. Applicability of the procedure:

This method is applicable to drinking, surface, and saline waters; domestic and industrial wastes.

a. Range of Concentration - The method is recommended for use in the range of 0.2 µg Hg/liter using a 100 ml sample; the upper limit can be varied by instrument expansion or dilution.

b. Pretreatment of the Sample - No pretreatment is necessary as the chemical digestion procedure inherent in the method is sufficient.

c. Treatment of interferences - High chlorides will require additional permanganate (as much as 25 ml). Then additional hydroxylamine sulfate is needed (25 ml) and the dead air space of the sample container swept out by air before addition of the stannous sulfate.

Some volatile organics can interfere but can be removed by a preliminary run without reagents.

d. Source of the Method - Manual of Methods for Chemical Analyses of Water and Wastes, 1974 ed., p. 119; U.S. EPA Technology Transfer, Cirti., OH 45268.

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WATER MONITORING PROCEDURE: Determination of Mercury Using the Flameless Atomic Absorption (Cold Vapor) Technique

Operating Procedures:

A. Equipment Preparation

B. Instrument Set-up

C. Reagent Preparation

D. Sample Handling and Preservation

E. Calibration

F. Sample Determination

G. Calculation

WATER MONITORING PROCEDURE: Determination of Mercury Using the Flameless Atomic Absorption (Cold Vapor) Technique

Equipment and Supply Requirements

A. Capital Equipment:

1. Atomic absorption spectrophotometer - Any commercial atomic absorption instrument is suitable if it has an open burner head area in which to mount an absorption cell, and if it provides the sensitivity and stability for the analyses. Also instruments designed specifically for the measurement of mercury using the cold vapor technique are commercially available and may be substituted.
2. Mercury hollow cathode lamp
3. Recorder - Any multi-range variable speed recorder that is compatible with the UV detection system is suitable.
4. Absorption cell - See Figure 4. The cell is constructed from glass or plexiglas tubing 25.4 mm O.D. x 114 mm (Note 1). The ends are ground perpendicular to the longitudinal axis and quartz window (25.4 mm diameter x 1.6 mm thickness) are cemented in place. Gas inlet and outlet ports (6.4 mm diameter) are attached approximately 12 mm from each end. The cell is strapped to a support and aligned in the light beam to give maximum transmittance.
5. Analytical balance, 200 gram capacity
6. Trip balance, 500 gram capacity
7. Water bath, capable of maintaining 95°C temperature

B. Reusable Supplies:

1. Air pump - Any peristaltic pump, with electronic speed control, capable of delivering 1 liter of air per minute may be used. (Regulated compressed air can be used in an open one-pass system.)
2. Six BOD bottles (plus one bottle is needed per sample)
3. Volumetric flasks
   - Six 1000 ml
   - Four 100 ml
   - One 250 ml
4. Pipets
   - Five 10 ml graduated
   - Two 1 ml graduated
   - One 1 ml volumetric
   - One 2 ml volumetric
   - Three 10 ml volumetric
   - One 5 ml volumetric
5. One 100 ml graduated cylinder; two 25 ml graduated cylinders
6. One Laboratory apron or coat
7. One pair safety glasses
8. One spatula
9. One pipet bulb
10. One wash bottle for distilled water
11. One glass stirring rod (about 6 inches long)

Note 1: An all glass absorption cell, 18 mm O.D. by 200 mm, with inlet 12 mm from the end, 18 mm O.D. outlet in the center, and with quartz windows has been found suitable.

Equipment and Supply Requirements (Continued)

12. One powder funnel
13. Rubber stoppers - two size #2 (for drying tube)
14. Fifteen feet of Tygon tubing
15. One glass tubing - 6 inches x 3/4 inch diameter
16. One Rotometer (any unit capable of measuring air flow of 1 liter/min.)
17. One set cork hole borers
18. One brush (for cleaning balance)

The following equipment is needed depending on which method is chosen to trap the mercury.

1. Liquid trap

    a. Straight glass frit, coarse porosity, such as Corning #404260
    b. Filtering flask, such as Corning #40058
    c. Rubber stopper, one hole to accept frit
    d. Reagents, $KMnO_4$ and H_2SO_4

2. Solid trap

    a. Activated carbon such as Barnebey and Cheney #580-13 or #580-22
    from: Barnebey and Cheney
    E. 8th Avenue & Cassidy Street
    Columbus, OH 43219

    or

    Coleman Instruments
    42 Madison St.
    Maywood, IL 60153
    Item #50-160

    b. Glassware - Can be assembled similar to the drying tube (Figure 3).

3. Closed System

    The following equipment is needed when using the closed system with a trap.

    a. Two position valve, or stopcock, such as Corning #442838
    b. Glass "Y" shaped tubing connecter
    c. Pinch clamp, type used for stopping flow in tubing

C. Consumable Supplies:

1. Sulfuric acid (H_2SO_4) concentrated
2. Nitric acid (HNO_3) concentrated
3. Potassium permanganate, $KMnO_4$
4. Potassium persulfate, $K_2S_2O_8$
5. Sodium chloride, NaCl

Equipment and Supply Requirements (Continued)

6. Hydroxylamine sulfate $(HONH_2) \cdot H_2SO_4$ or
   Hydroxylamine hydrochloride $NH_2OH \cdot HCl$
7. Stannous sulfate, $SnSO_4$ or stannous chloride, $SnCl_2$
8. Mercuric Chloride, $HgCl_2$
9. Hydrochloric acid concentrated
10. Magnesium perchlorate, $Mg(ClO_4)_2$ for drying tube, 20 g.
11. Distilled water
12. Sponges (for cleaning laboratory table tops)
13. Notebook for recording weights and readings
14. Two pieces of glass tubing (5 mm diameter, about two inches long) for the drying tube
15. Glass wool (for drying tube)
16. Plastic weighing boats (about 10)
17. Pen or pencil

WATER MONITORING PROCEDURE: Determination of Mercury Using the Flameless Atomic Absorption (Cold Vapor) Technique

Sample

Chemical Sample Preparation

a. Oxidation of all mercury to mercuric form
b. Reduction of all mercuric mercury to metallic mercury

Aeration

The metallic mercury is ciruculated as a vapor through the system

Flameless Atomic Absorption

Absorption of energy at 253.7 nm from a hollow cathode lamp measured by a photodetector

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Cleaning of Glassware	1. Wash with detergent.	1a. Cleaning should be carried out in this order. 1b. Care should be taken to insure clean glassware. 1c. If possible glassware should be reserved for mercury analysis only and separated from other glassware.	
	2. Rinse with tap water.		
	3. Rinse with 1:1 nitric acid.	3a. Add 500 ml concentrated nitric acid (HNO_3) to 500 ml distilled water.	
	4. Rinse with tap water.		
	5. Rinse with 1:1 hydrochloric acid.	5a. Add 500 ml concentrated hydrochloric acid (HCl) to 500 ml distilled water.	
	6. Rinse with tap water.		
	7. Rinse with distilled water.		
2. Balance Preparation	1. Check all balances for cleanliness and proper operation.		
B. Instrumental Set-up			
1. Flow System	1. Before operation of the instrument, four additions to the system should be considered (Figure 1).	1a. There are two ways the flow system can be set up. It can be operated as a closed or open system. In the closed system the mercury vapor continously passes through the system until wasted in the mercury trap by the operator. In the open system the vapor passes through the absorption tube only once and goes directly to the trap. Which system is chosen will dictate what equipment is necessary. Figure I shows the choices and the equipment necessary for each.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Instrumental Set-up (Continued) 2. Mercury Trap - Liquid Type	1. One of the following mercury traps should be included in the system.	1a. Because of the toxic nature of mercury vapor, precaution must be taken to avoid contamination. The vapor will be held in the trap after it has been measured.	
	2. For a liquid type trap, use a 250 ml side arm filtering flask.	2a. Use a filtering flask such as Corning #400580 or its equivalent.	
	3. Assemble as shown in Figure 2.	3a. Use a #3 cork hole borer to make the hole.	
	4. Insert straight gas dispersion tube or frit through the hole so that the bottom or fritted end is about one inch above bottom of the flask.	4a. Frit should have a coarse porosity such as Corning #404260 or equivalent. The frit should always fall below liquid level in the flask. Should the level become low add more liquid (Reagent #10). The nonfritted end should be lubricated and care taken when the frit is inserted through the stopper so as not to break the frit and injure the worker.	
	5. Insert into filtering flask.		
	6. Connect tygon tubing to top end of frit and a second piece of tygon tubing to the side arm of filtering flask.	6a. Care should be taken so that the liquid level does not come close to the opening of the side arm of the flask. This could flood the instrument if allowed to do so. If flooding should occur, dismantle the absorption tube and clean it and the tubing immediately.	
	7. Add 200 ml of 1:1 potassium permanganate ($KMnO_4$) - sulfuric acid (H_2SO_4) Reagent (Reagent #10).	7a. A solution of 0.25% iodine in a 3% potassium iodide (KI) solution may also be used (Reagent 12). 7b. Filling the flask can be postponed until all of the apparatus is assembled.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTE
B. Instrumental Set-up (Continued)			
2. Mercury Trap - Solid Type	1. The apparatus can be prepared similar to the drying tube (B-3) but packed with 2-3 grams of activated carbon.	1a. Locate after 2 position valve in closed system, Figure 1 (system two) or after the analyzer in an open system, Figure 1 (system three).	
	2. The equipment can be purchased with adsorbent as an option from the analyzer manufacturer.	2a. Position as above.	
3. Drying Tube	1. Construct as shown in Figure 3.	1a. Place between sample container and instrument.	
	2. Bore a hole through a number 2 stopper with a number 2 cork hole borer. Repeat with a second stopper.		
	3. Insert a 2 inch long piece of glass tubing (5 mm diameter) through each stopper allowing about 1/2 inch protruding from each end.	3a. Care should be taken when inserting glass tubing.	
	4. Fill a 6 inch piece of 3/4 inch diameter tubing with 20 grams of magnesium perchlorate ($Mg(ClO_4)_2$).	4a. Other drying agents such as calcium chloride ($CaCl_2$) may be used.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Instrumental Set-up (Continued)	5. Use a small piece of glass wool in each end of the tube to prevent loss of granules.	5a. The tube should not be packed so tight as to restrict gas flow.	
	6. Insert stopper prepared above in each end of tube.		
	7. Replace drying agent when needed.	7a. Replace magnesium perchlorate or any drying agent regularly. These materials tend to cake and form a plug when their limit of saturation is approached. The length of time the material will last will vary with use and samples. Experience will dictate a routine.	
4. Rotometer	1. Must be capable of measuring a gas flow of 1 liter per minute.	1a. Place between water trap and instrument. See Figure 1 for location. 1b. The rotometer may be removed from the circuit after the instrument pump rate is checked. 1c. The flow rate should be checked periodically to insure flow rate has not changed.	
	2. Connect one length of tubing between the sample container and the drying tube through the rotometer to the fitting of the instrument.	2a. The connection must be made to the sample container by side arm. Reverse tubing connections may flood the instrument with liquid.	
	3. A second length of tubing should begin at the out fitting of the instrument and proceed to the next piece of equipment.	3a. See Figure 1 for gas flow path.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Instrumental Set-up (Continued)			
5. Two Position Valve	1. A two position valve is necessary when using a closed system and a trap. Use stopcock, Corning No. 442838 or equivalent for the two way valve or stopcock.		
	2. One position of the valve should go through the trap to the sample container. The other position should by-pass the trap and be connected to the frit of the sample container.	2a. It is important to maintain a specific air volume in the system. Once the system is calibrated, this volume cannot be changed unless the system is recalibrated.	
6. Recorder (Optional)	1. Any multi-range variable speed recorder that is compatible with the equipment is suitable.	1a. Use of a recorder or its equivalent for analysis of potable water samples is strongly recommended.	
7. Instrument	1. Follow instrument manufacturer's directions.		
C. Reagent Preparation			
1. Sulfuric Acid 0.5 N	1. Add 14.0 ml concentrated sulfuric acid (H_2SO_4) to approximately 500 ml water and mix. Then dilute with water to 1 liter volume.	1a. The concentrated H_2SO_4 should be of low mercury concentration. 1b. Unless specified the term water means distilled water. 1c. Use a 25 ml graduated cylinder to measure the sulfuric acid.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
2. Potassium Permanganate Solution - 5% Solution w/v	1. Prepare 100 ml of solution containing 5.0 grams potassium permanganate ($KMnO_4$).	1a. Should a larger amount of reagent solution be needed the same ratio should be maintained. For example: prepare 1000 ml of solution containing 50 grams $KMnO_4$. 1b. Weigh out in plastic weighing boat on a trip balance.	
3. Potassium Persulfate Solution - 5% Solution w/v	1. Dissolve 5.0 g potassium persulfate ($K_2S_2O_8$) in water and dilute to 100 ml.	1a. Weigh out in a plastic weighing boat on a trip balance.	
4. Sodium Chloride - Hydroxylamine Sulfate Solution Solution 12% NaCl 12% $((HONH_2)_2 \cdot H_2SO_4)$ $\}$ w/v	1. Dissolve 12.0 g of sodium chloride (NaCl) and 12.0 g of hydroxylamine sulfate ($(HONH_2)_2 \cdot H_2SO_4$) in water and dilute to 100 ml.	1a. Hydroxylamine hydrochloride ($NH_2OH \cdot Cl$) may also be used. It should be prepared in the same manner. 1b. Weigh out in a plastic weighing boat on a trip balance.	
5. Stannous Sulfate Solution - 10% Solution w/v	1. Add 25.0 g stannous sulfate ($SnSO_4$) to about 40 ml 0.5 N sulfuric acid and dilute with 0.5 sulfuric acid to 50 ml.	1a. Stannous chloride ($SnCl_2 \cdot 2H_2O$) may be used and be prepared in the same manner. 1b. The acid is reagent no. 1. 1c. This is a suspension and should be stirred continuously during use. 1d. Weigh out in a plastic weighing boat on a trip balance.	
6. Sulfuric Acid Concentrated (H_2SO_4)	1. No preparation necessary.	1a. This should be reagent grade and low in mercury concentration. 1b. Caution: this is corrosive.	

WATER MONITORING PROCEDURE: Determination of Mercury Using the Flameless Atomic Absorption (Cold Vapor) Technique

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
7. Nitric Acid Concentrated (HNO_3)	1. No preparation necessary.	1a. This should be reagent grade and low in mercury content. 1b. Caution: this is corrosive. 1c. If a high reagent blank is obtained, it may be necessary to distill the nitric acid.	
8. Stock Mercury Solution ($HgCl_2$)	1. Dissolve 0.1354 g of mercuric chloride ($HgCl_2$) in water.	1a. Weigh out in a plastic weighing boat on an analytical balance.	
	2. Add 10 ml concentrated nitric acid (HNO_3).	2a. Caution: solution will increase in temperature. 2b. Use a 10 ml graduated pipet.	
	3. Cool to room temperature.		
	4. Dilute to 100 ml with water.	4a. Concentration of stock solution is now 1 ml = 1 mg Hg. 4b. Stock solution is stable for several months.	
9. Intermediate Mercury Solution - ($HgCl_2$) Dilution of Solution 8	1. The intermediate solution is a dilution of the stock solution to adjust the concentration of Hg to 0.1 µg/ml. Proceed as follows.	1a. Prepare fresh before use.	
	2. Add about 700 ml water to a 1000 ml volumetric flask.		
	3. Add 0.5 ml concentrated HNO_3.	3a. The nitric acid concentration of the dilutions including the working solution should be maintained at 0.15%. This acid should be added to the flask before addition of the aliquot. 3b. Use a 1 ml pipet graduated in tenths.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)	4. Add 10 ml stock Hg solution.	4a. Use a 10 ml volumetric pipet.	
	5. Dilute to 1000 ml mark. This solution contains 10 μg/ml.		
10. Working Mercury Solution ($HgCl_2$)	1. Add about 700 ml water to a 1000 ml volumetric flask.		
Dilution of Solution 9	2. Add 1.5 ml concentrated HNO_3.	2a. Use a 10 ml graduated pipet.	
	3. Add 10 ml of intermediate solution (10 μg/ml).	3a. Use a 10 ml volumetric pipet.	
	4. Dilute to 1000 ml mark. This is working solution and contains 0.1 μg/ml.		
Prepare 11 or 12 for a liquid trap.			
11. Potassium Permanganate 0.1 M ($KMnO_4$) and	1. Dissolve .316 g potassium permanganate ($KMnO_4$) in 100 ml water.	1a. Let stand until following solution is prepared. 1b. Weigh out in a plastic weighing boat or a trip balance.	
Sulfuric Acid 10% Solution (for Mercury Trap)	2. Add 10 ml concentrated sulfuric acid (H_2SO_4) to about 80 ml water. Dilute to 100 ml with water.	2a. Caution: heat generated. 2b. Should be at room temperature before volume adjustment. 2c. Use a 10 ml graduated pipet.	
	3. Mix equal volumes of each solution; $KMNO_4$ (1) and H_2SO_4 (2).		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
12. Iodine (0.25) in KI (3%) Solution	1. Measure 250 ml of distilled water in a graduated cylinder.		
	2. Transfer about half the water to a 500 ml Erlenmeyer flask.		
	3. Weigh out 7.5 grams of potassium iodide (KI).	3a. A trip balance can be used.	
	4. Transfer the potassium iodide to the 500 ml Erlenmeyer flask and dissolve.		
	5. Weigh out 0.63 grams of iodine (I).	5a. A trip balance can be used.	
	6. Add to the Erlenmeyer flask and dissolve.		
	7. Add the remainder of the water.		
	8. Mix well.		
D. Sample Handling and Preservation	1. Upon collection, the sample pH should be lowered to 2 or lower by the addition of concentrated nitric acid (HNO_3).	1a. If only dissolved mercury is to be determined, the sample should be filtered before addition of the acid. For total mercury the filtration is omitted. 1b. If nitric acid cannot be used because of shipping restrictions, the sample may be initially preserved by icing and immediately shipped to the (Continued)	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>D. Sample Handling and Preservation</td><td></td><td>laboratory. Upon receipt in the laboratory, the sample must be acidified with concentrated nitric acid to pH<2.</td><td></td></tr>
<tr><td></td><td>2. Collect samples in appropriate container.</td><td>2a. Samples should be collected in acid-washed glass or high density polyethylene bottles.</td><td></td></tr>
<tr><td></td><td>3. Do not store samples beyond the acceptable time.</td><td>3a. Samples should be analyzed within 38 days if collected in glass bottles and within 13 days if collected in polyethylene bottles.</td><td></td></tr>
<tr><td>E. Calibration</td><td>1. Prepare a series of standards from the working mercury standard.</td><td>1a. Prepare as follows:
<table>
<tr><th>ml's of Standard</th><th>ml's of Water</th><th>Concentrated µgHg</th></tr>
<tr><td>0.0</td><td>100.0</td><td>0.00</td></tr>
<tr><td>0.5</td><td>99.5</td><td>0.05</td></tr>
<tr><td>1.0</td><td>99.0</td><td>0.10</td></tr>
<tr><td>2.0</td><td>98.0</td><td>0.20</td></tr>
<tr><td>5.0</td><td>95.0</td><td>0.50</td></tr>
<tr><td>10.0</td><td>90.0</td><td>1.00</td></tr>
</table>
1b. Use 300 ml BOD bottles.
1c. Use volumetric pipets to pipet the standards.
1d. A 100 ml graduated cylinder may be used for the water.</td><td></td></tr>
<tr><td></td><td>2. Mix thoroughly.</td><td></td><td></td></tr>
<tr><td></td><td>3. Add 5 ml concentrated sulfuric acid (H_2SO_4) to each bottle.</td><td>3a. Mix thoroughly.
3b. Use caution when using concentrated acids.
3c. Use a 5 ml graduated pipet.</td><td>I.E.3
VII.E.3
(p. 22 & p. 26)</td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
12. Iodine (0.25) in KI (3%) Solution	1. Measure 250 ml of distilled water in a graduated cylinder.		
	2. Transfer about half the water to a 500 ml Erlenmeyer flask.		
	3. Weigh out 7.5 grams of potassium iodide (KI).	3a. A trip balance can be used.	
	4. Transfer the potassium iodide to the 500 ml Erlenmeyer flask and dissolve.		
	5. Weigh out 0.63 grams of iodine (I).	5a. A trip balance can be used.	
	6. Add to the Erlenmeyer flask and dissolve.		
	7. Add the remainder of the water.		
	8. Mix well.		
D. Sample Handling and Preservation	1. Upon collection, the sample pH should be lowered to 2 or lower by the addition of concentrated nitric acid (HNO_3).	1a. If only dissolved mercury is to be determined, the sample should be filtered before addition of the acid. For total mercury the filtration is omitted. 1b. If nitric acid cannot be used because of shipping restrictions, the sample may be initially preserved by icing and immediately shipped to the (Continued)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Calibration (Continued)	4. Add 2.5 ml concentrated nitric acid (HNO_3) to each bottle.	4a. Mix thoroughly. 4b. Use caution when using concentrated acids. 4c. Use a 5 ml graduated pipet.	
	5. Add 15 ml potassium permanganate ($KMnO_4$) (Reagent no. 2).	5a. Shake and add additional potassium permanganate solution, if necessary, until the purple color persists for the following standing time. 5b. Use a 25 ml graduated cylinder for the addition.	
	6. Allow to stand 15 minutes.		
	7. Add 8 ml potassium persulfate ($K_2S_2O_8$) (Reagent no. 3).	7a. Use a 10 ml graduated pipet.	
	8. Heat in a water bath at 95°C for 2 hours.	8a. The heat step is required for organic mercury compounds. For standards prepared with distilled water and mercuric chloride, the heating step is not necessary.	
	9. Cool to room temperature.		
	10. Add 6 ml of the sodium chloride-hydroxylamine sulfate ($NaCl \cdot (HONH_2)_2 \cdot H_2SO_4$) (Reagent no. 4).	10a. Shake well. 10b. Use a 10 ml graduated pipet. 10c. This reagent should decolorize the solution. If 6 ml is not enough, add sufficient extra to complete the decolorization.	
	11. Allow to stand at least 30 seconds.	11a. Up to this point all samples to be run can be treated as a group. From this point each must be done individually as the mercury is liberated immediately upon addition of the stannous sulfate.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Calibration (Continued)	12. Add 5 ml of stannous sulfate suspension ($SnSO_4$) (Reagent no. 5).	12a. Use a 5 ml graduated pipet with a large bore opening.	
	13. Immediately insert bubbler into the bottle.	13a. The air mover (pump-air, cylinder, etc.) should be calibrated before use at 1 liter/minute. It should be on and allowed to operate continously when analyzing samples or standards. 13b. The reading will increase and reach a maximum within one minute.	
	14. Observe reading.	14a. When a recorder is used, allow the pen to level off.	
	15. Trap the mercury.	15a. In the closed system the valve must be turned to the trap position until the absorbance returns to the minimum value. The aeration should be continued. In the open system wait until the absorbance returns to the minimum value.	VII.E.15 (p. 26)
	16. Continue with the remaining standards.	16a. Repeat steps 12-15, Section D.	
	17. Plot a standard curve.	17a. Plot the peak height (from the recorder plot) versus the concentration in micrograms of mercury on arithmetic paper.	II.E.17a (p. 24)
F. Sample Determination	1. Transfer 100 ml of the sample to a 300 ml BOD bottle.	1a. The sample should not contain more mercury than the upper limit of the equipment being used. The range of the method may be varied through instrument and/or recorder expansion and by using a larger volume of sample. 1b. The usual range of the procedure is 0.2 to about 10.0 µg Hg/liter (.02 to 1.0 µgHg/100 ml).	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Determination (Continued)	2. Treat sample(s) with same procedure used in calibration section steps 3 through 15.	2a. The standard curve once plotted should be verified by use of at least a reagent blank and one standard at or near the concentration of interest either daily or with each batch run.	
G. Calculations	1. Determine the peak height of the unknown(s) from the recorder chart and read the mercury value from the standard curve.	1a. As constructed under step 17, Operating Procedure E, Calibration.	
	2. Calculate the mercury concentration in the sample.	2a. The value is expressed as μgHg/liter so: $\mu gHg/liter = \frac{\mu gHg \text{ in the sample}}{\text{volume}} \times \frac{1000}{\text{vol. of sample}}$ Example: If the sample was diluted by adding 50 ml of sample and adding 50 ml of distilled water and a value obtained showing 0.1 μgHg then: $\frac{\mu gHg}{liter} = 0.1\ \mu gHg \times \frac{1000}{50\ ml} = \frac{2.0\ \mu gHg}{liter}$	
	3. Report mercury concentration as follows: Below 0.2 μg/l as <0.2 μg/l Between 1.0 and 10.0 μg/l using one place after decimal Above 10.0 μg/l using only whole numbers	4a. < = less than.	

WATER MONITORING PROCEDURE: Determination of Mercury Using the Flameless Atomic Absorption (Cold Vapor) Technique

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

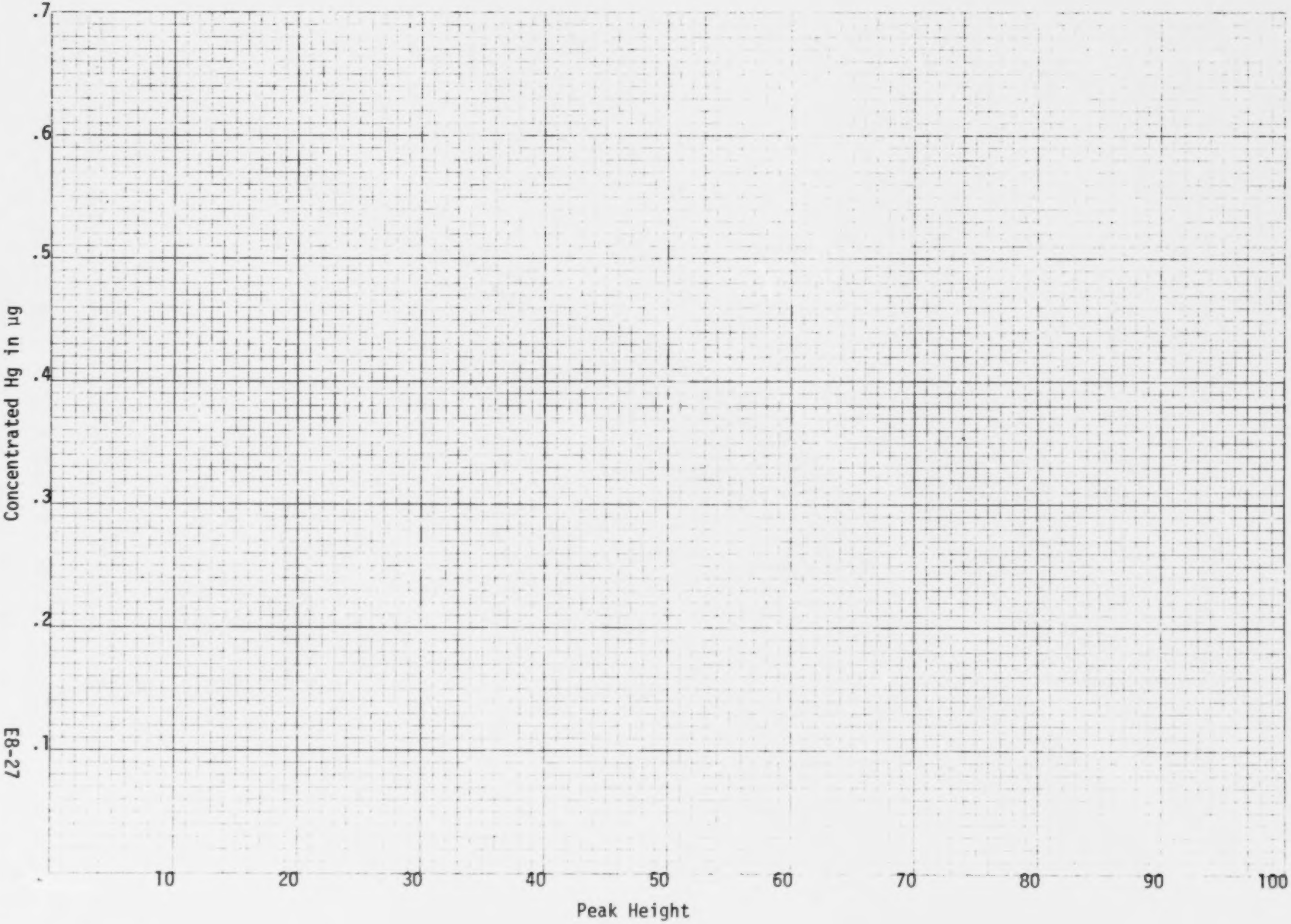
Training Guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

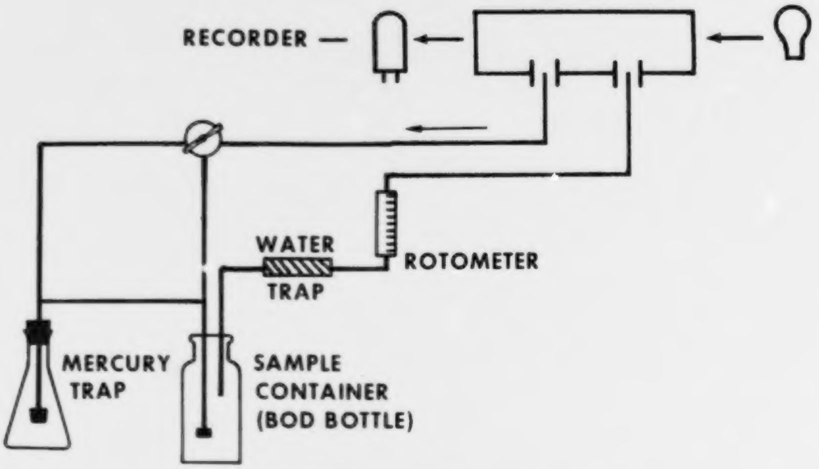
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.3	Theoretical Concepts The method is based on the method developed by Hatch and Ott. The mercury in the sample is oxidized to the mercuric ion with potassium permanganate in a nitric-sulfuric acid medium. Hydroxylamine sulfate is then added to remove the excess permanganate. Stannous sulfate, is then added to reduce the mercury to metallic form. Then the mercury is vaporized and circulated by the bubbler system. This consists of a circulating pump and the bubbler. Measurement is made with a flameless atomic absorption spectrophotometer. The energy at the 253.7 nm mercury line emitted by a mercury lamp is absorbed by the mercury vapor in the flow-through absorption cell. The change in transmittance is detected by a phototube.	Analytical Chemistry, vol. 40, p. 2085, December 1968.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.17a	The standard curve is a reference to a fundamental law of absorption chemistry known as the Beer-Lambert law. Simply, this law states that the amount of energy absorbed by a solution is proportional to the concentration of the absorbing material in the solution. Applied to this outline the amount of energy absorbed at the wavelength of 253.7 nm is proportional to the amount of mercury present in a solution. If the concentrations of a series of known solutions (prepared as in step 1 under Calibration) and peak height are plotted, a straight line should result. When an unknown sample value is obtained, its mercury content can be determined from the straight line or standard curve. To use the attached graph paper prepare the standards as in step 1 of the Calibration section and run them as described. The known concentrations are plotted along the bottom of the graph. Then plot the peak heights obtained from the recorder chart plotted on the left of the graph. Where the known concentration line intersects with the appropriate peak height, a mark is made. After all six standards are plotted, draw a line through the marks.	

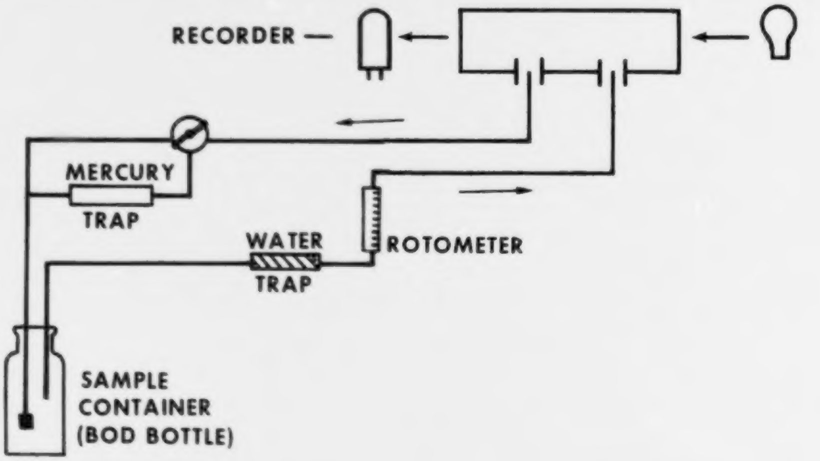
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.6.1a	The criteria for certification of a laboratory for analysis of drinking water samples for compliance with the Safe Drinking Water Act (93-523) lists as mandatory equipment a recorder to be used when analyzing for mercury.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.3	Loss of mercury may occur at elevated temperature. However, with the stated amounts of acid the temperature rise is only about 13°C (25-38°C) and no losses of mercury will occur.	EPA Method Study 8, Total Mercury in Water, EPA-600/4-77/012, Feb. 1977.
E.15	Be sure to close the valve before running another sample/standard when using the closed system. Since the system is being calibrated with the valve in that position, running a sample/standard in the open mode will produce a nonacceptable value. This is due to the change in volume of the system. Operation in the open mode does not require the valve. Consequently, the opening and closing will not have to be done.	

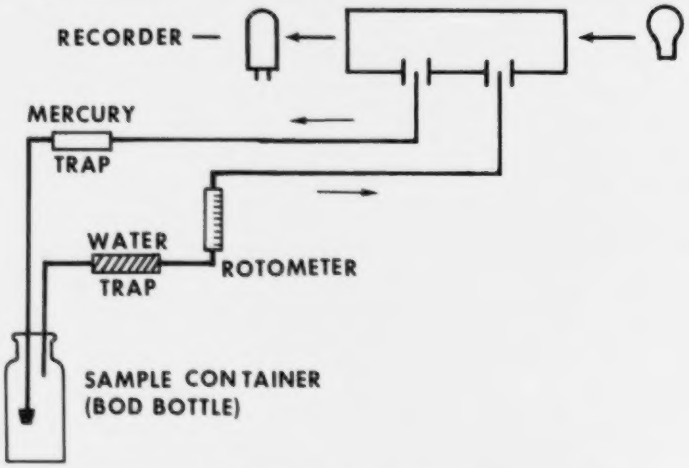

.7
.6
.5
.4
.3
.2
.1
Concentrated Hg in µg
10
20
30
40
50
60
70
80
90
100
Peak Height


RECORDER
WATER
TRAP
ROTOMETER
MERCURY
TRAP
SAMPLE
CONTAINER
(BOD BOTTLE)

SYSTEM ONE: LIQUID MERCURY TRAP CLOSED SYSTEM

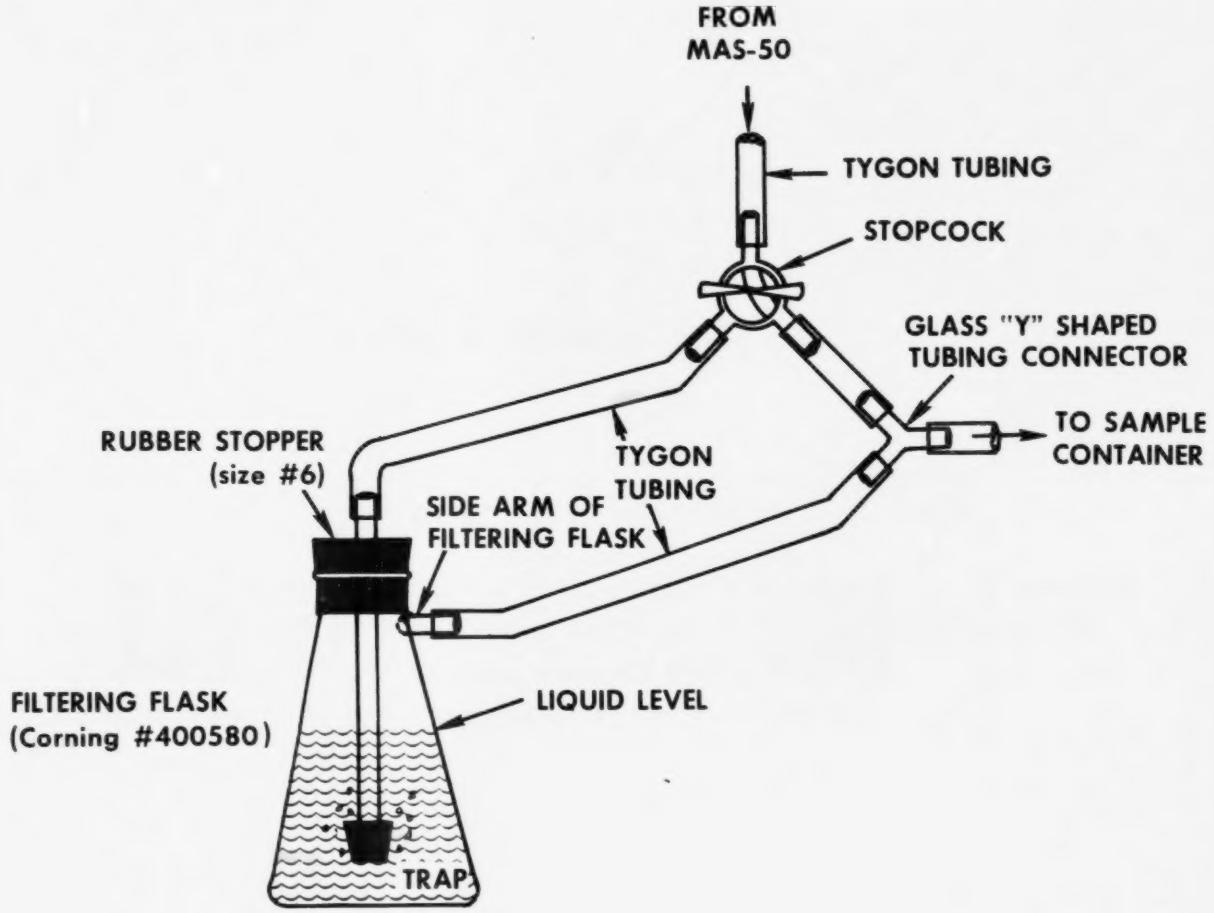

RECORDER
MERCURY
TRAP
WATER
TRAP
ROTOMETER
SAMPLE
CONTAINER
(BOD BOTTLE)

SYSTEM TWO: SOLID MERCURY TRAP CLOSED SYSTEM


RECORDER
MERCURY
TRAP
WATER
TRAP
ROTOMETER
SAMPLE CONTAINER
(BOD BOTTLE)

SYSTEM THREE: SOLID MERCURY TRAP OPEN SYSTEM

Figure 1. FLOW SYSTEMS FOR THE COLD VAPOR TECHNIQUE FOR MERCURY



FROM
MAS-50
TYGON TUBING
STOPCOCK
GLASS "Y" SHAPED
TUBING CONNECTOR
TO SAMPLE
CONTAINER
RUBBER STOPPER
(size #6)
TYGON
TUBING
SIDE ARM OF
FILTERING FLASK
FILTERING FLASK
(Corning #400580)
LIQUID LEVEL
TRAP

FIGURE 2. ARRANGEMENT OF TWO-POSITION STOPCOCK AND MERCURY TRAP

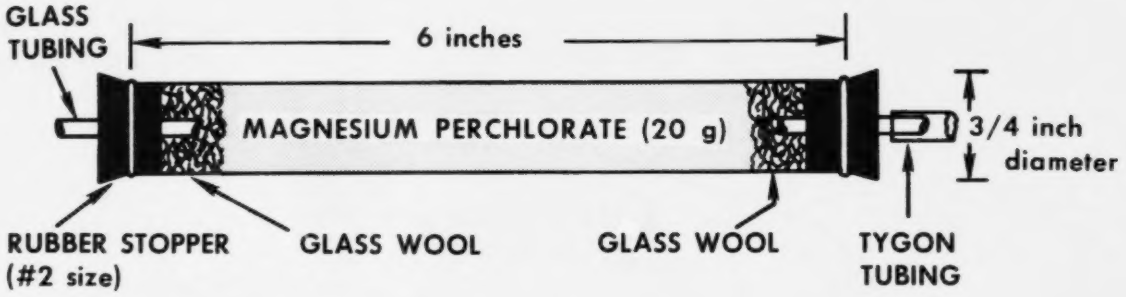

GLASS TUBING
6 inches
MAGNESIUM PERCHLORATE (20 g)
3/4 inch diameter
RUBBER STOPPER (#2 size)
GLASS WOOL
GLASS WOOL
TYGON TUBING

FIGURE 3. DRYING TUBE

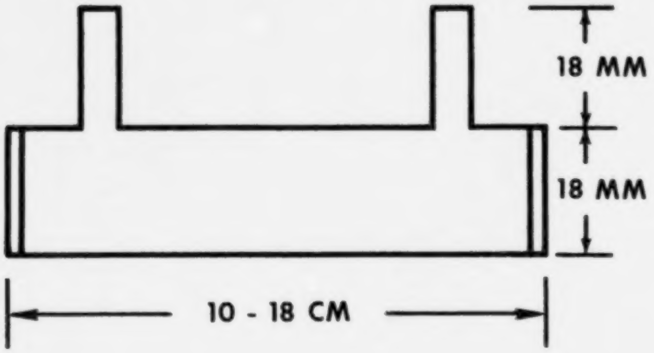


FIGURE 4. CELL FOR MERCURY MEASUREMENT BY COLD VAPOR TECHNIQUE

The length and OD of the cell are not critical. The body of the cell may be of any tubular material but the end windows must be of quartz because of the need for UV transparency.

The length and diameter of the inlet and outlet tubes are not important, but the position of the side arms may be a factor in eliminating recorder noise. There is some evidence that displacement of the air inlet arm away from the end of the cell results in smoother readings. A mild pressure in the cell can be tolerated, but too much pressure may cause the glued-on end windows to pop off.

Cells of this type may be purchased from various supply houses.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF ARSENIC AND SELENIUM

as applied in

DRINKING WATER TREATMENT FACILITIES
and in the
DISTRIBUTION SYSTEMS OF DRINKING WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

CH.ME.lab.WMP.2.11.77

WATER MONITORING PROCEDURE: Determination of Arsenic and Selenium

General Description of Equipment and Supplies Used in the Process

A. Capital Equipment:

1. Balance, analytical - sensitivity 0.1 milligrams
2. Atomic absorption spectrophotometer - see instrument section
3. pH meter
4. Hot plate, 110 V
5. Magnetic stirrer
6. Pan balance

B. Reusable Supplies:

1. Flasks, volumetric, 50 ml, 100 ml, 1000 ml
2. Flasks, Erlenmeyer, 250 ml
3. Pipet, volumetric, 25 ml 50 ml
4. Pipet, micro, 1 ml graduated 0.1 ml
5. Pipet, measuring, 1 ml, 10 ml
6. Graduated cylinders, 500 ml, 100 ml, 50 ml, 25 ml
7. Beakers, 250 ml
8. Funnel, 80 mm diameter
9. Ring stand and 3 inch ring
10. Watch glass
11. Anion and cation exchange resin cartridges
12. Reagent bottles
13. pH paper
14. Specialized glassware - See apparatus section

C. Consumable Supplies:

1. Reagents, analytical reagent grade

   a. Arsenic trioxide
   b. Selenium metal
   c. Zinc metal (200 mesh)
   d. Potassium iodide
   e. Stannous chloride
   f. Sulfuric acid 18N
   g. Hydrochloric acid, concentrated
   h. Nitric acid
   i. Perchloric acid, 70-72 percent
   j. Sodium hydroxide

2. Gases

   a. Argon
   b. Hydrogen

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WATER MONITORING PROCEDURE: Determination of Arsenic and Selenium

1. Analysis Objective:

   To determine the arsenic and selenium concentration, as listed in the Interim Primary Drinking Water Regulations, in water samples from potable water treatment and distribution facilities.

2. Brief Description of the Analysis:

   Samples are prepared to distinguish between inorganic and total (inorganic and organic) metal by appropriate acid digestion. Either one of the prepared samples or standards are treated with $SnCl_2$, a reducing agent to convert the metal to its lowest oxidation state, Zinc is added to the acidified sample, generating hydrogen and producing the evolution of the metal hydride which is aspirated into a Argon-Hydrogen flame of an atomic absorption spectrophotometer. Calculations are made from a standard curve by measuring the peak heights of the samples and reading the concentration.

3. Applicability of this Procedure:

   a. Range of Concentration - From 2 to 20 µg/l is the working range of the method.
   b. Pretreatment of Samples - Upon collection the pH of the sample should be lowered to below 2 by the addition of concentrated nitric acid. The maximum holding time is 6 months.
   c. Treatment of Interferences - In analyzing most surface and ground waters, interferences are rarely encountered. Organic forms of arsenic and selenium must be oxidized before analysis.

   d. Source of Procedure - Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Monitoring and Supply Laboratory, Cincinnati, Ohior.

WATER MONITORING PROCEDURE: Determination of Arsenic and Selenium

Operating Procedures:

A. Apparatus and Instrumentation

B. Instrument Calibration

C. Reagent Preparation

D. Sample Preparation

E. Standards Preparation

F. Samples and Standards Treatment

G. Calculations

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Apparatus and Instrumentation	1. Atomic absorption spectrophotometer.	1a. Any atomic absorption spectrophotometer that allows the introduction of a gaseous sample; double-beamed instrument preferred because of increased stability.	
	2. Hollow cathode lamp.	2a. Arsenic or selenium hollow cathode lamp compatible with the spectrophotometer.	
	3. Recorder.	3a. Any variable-speed recorder that is compatible with the spectrophotometer; a chart speed of 10 mm/minute recommended.	
	4. Flowmeter.	4a. A flowmeter capable of measuring 1 l/minute, such as a Gilmont No. 12 or equivalent used for auxiliary argon.	
	5. Medicine dropper.	5a. A dropper capable of delivering 1.5 ml, fitted into a size "0" rubber stopper.	
	6. Reaction flask.	6a. A pear-shapped vessel with side arm and 100 ml capacity, both arms having 14/20 joint (Scientific Glass JM-5835) or equivalent.	
	7. Special gas inlet-outlet tube.	7a. Constructed from a micro cold finger condenser (Scientific Galss JM-3325) or equivalent by cutting off the portion below the 14/20 ground glass joint.	
	8. Magnetic stirrer.	8a. Must be strong enough to homogenize a 50% mixture of zinc dust and water. 8b. See reagent section.	
	9. Drying tube.	9a. 100 mm long polyethylene tube filled with glass wool to keep particulate matter out of the burner.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Apparatus and Instrumentation (Continued)	10. Apparatus setup: See schematic.	10a. Connect the apparatus with the burner of the spectrophotometer as indicated in the schematic. Connect the outlet of the reaction vessel to the auxiliary oxidant input of the burner with Tygon tubing. Connect the inlet of the reaction vessel to the outlet side of the auxiliary oxidant, argon supply, control valve of the instrument.	
B. Instrument Calibration	1. Prepare atomic absorption spectrophotometer for operation.	1a. It is not possible to formulate instructions applicable to every instrument but in general one can follow the step sequence in the adjacent column and by consulting the instruction manual for your particular instrument.	
	2. Install the hollow cathode lamp for the element being measured (As or Se) in the instrument, set the wavelength dial and align the lamp in accordance with the manufacturer's instructions.	2a. Arsenic wavelength 193.7 mm. Selenium wavelength 196.0 mm. 2b. See instruction manual for your particular instrument.	
	3. Set the slit width according to the manufacturer's suggested setting for the element being measured (As or Se).		
	4. Turn on the instrument and ajust the current to the hollow cathode lamp as suggested by the manufacturer.	4a. Allow the instrument to warm up, usually 10-20 minutes, until the energy source stabilizes.	

WATER MONITORING PROCEDURE: Determination of Arsenic and Selenium

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Instrument Calibration (Continued)	5. Install a Boling burner head.		
	6. Turn on the argon and adjust to a flow rate of about 8 l/minute with the auxiliary argon flow at 1 l/minute.		
	7. Turn on the hydrogen, adjust the flow rate of about 7 l/minute and ignite the flame which will be essentially colorless.	7a. The gas flow rates may require adjustment to optimize the flame for your particular instrument	
	8. Adjust the burner head both sideways and vertically in the light path until maximum response is obtained by atomizing a freshly prepared standard solution for the element being measured (1.00 ml equals 1.00 mg (As or Se))	8a. The instrument is now ready to run standards and samples.	
C. Reagent Preparation 1. Dionized Distilled Water	1. Prepare by passing distilled water through a mixed bed of cation and anion exchange resin.	1a. Use deionized distilled water for the preparation of all reagents, calibration standards, and as dilution water.	
2. Nitric Acid Concentrated (HNO_3)	1. Commercially available reagent grade.	1a. If metal impurities are found to be present, distill reagent grade nitric acid in a borosilicate glass distillation apparatus.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)			
3. Hydrochloric Acid	1. Commercially avialagle reagent grade.		
4. Diluent - Stock	1. Add 100 ml 18 N sulfuric acid and 400 ml hydrochloric acid to 400 ml deionized distilled water in a 1 liter volumetric flask and bring to volume with deionized distilled water.	1a. Use a 100 ml and 500 ml graduate. 1b. Diluent used for preparation of working standards.	
5. Potassium Iodide Solution	1. Weigh 20 grams potassium iodide, KI, on a pan balance.	1a. Use a weighing disk.	
	2. Transfer the reagent into a 250 ml Erlenmeyer flask and dissolve with 100 ml deionized distilled water.	2a. Use a 100 ml graduate.	
6. Stannous Chloride Solution	1. Weigh 100 grams stannous chloride, $SnCl_2$, on a pan balance.	1a. Use a weighing disk.	
	2. Transfer the reagent into a 250 ml Erlenmeyer flask and dissolve with 100 ml concentrated hydrochloric acid, HCL.	2a. Use a 100 ml graduate.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued) 7. Stock Arsenic	1. Weigh 4 grams sodium hydroxide, NaOH, on a pan balance.	1a. Use a weighing disk.	
	2. Transfer the reagent to a 250 ml beaker and add 100 ml of deionized water, allow to dissolve.	2a. Stir if necessary with a glass or plastic rod.	
	3. Weigh 1.3209 grams arsenic trioxide, As_2O_3 on an analytical balance.	3a. Using a weighing disk.	
	4. Transfer the reagent to the beaker containing the NaOH solution, allow to dissolve.	4a. Stir if necessary.	
	5. After dissolution transfer into a clean 1000 ml volumetric flask and dilute to the mark with deionized distilled water.	5a. Use a plastic wash bottle to rinse the beaker and stirring rod into the volumetric flask. 5b. One ml equals 1.00 mg As (1000 mg/1).	
8. Intermediate Arsenic Solution	1. Pipet 1 ml stock arsenic solution (1000 mg/1) into a 100 ml volumetric flask and bring to volume with deionized distilled water containing 1.5 ml concentrated nitric acid per liter.	1a. One ml equals 10.0 mg As (10 mg/1). 1b. Use a volumetric pipet. 1c. This solution is made up fresh at time of use.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued) 9. Standard Arsenic Solution	1. Pipet 10 ml intermediate arsenic solution in 100 ml volumetric flask and bring to volume with deionized distilled water containing 1.5 ml concentrated nitric acid per liter.	1a. One ml equals 1 µg As (1 mg/l). 1b. Use a volumetric pipet. 1c. This solution is made up fresh at time of use.	
10. Perchloric Acid 70-72% $HClO_4$	1. Commercially available grade.		
D. Sample Preparation 1. Inorganic Arsenic or Selenium	1. To a 50 ml volumetric flask add 25 ml of water sample, 20 ml concentrated HCl and 5 ml of 18N H_2SO_4.	1a. Allow to cool to ambient temperature. 1b. Use a 25 ml volumetric pipet. 1c. Use 10 ml measuring pipets.	
2. Total (Inorganic and Organic) Arsenic	1. To 50 ml of water sample in a 150 ml beaker add 10 ml of concentrated nitric acid, and 12 ml of 18 N sulfuric acid. This mixture is evaporated to SO_3 fumes (a volume of about 20 ml).	1a. Oxidizing conditions must be maintained at all times to avoid loss of arsenic.	
	2. To maintain oxidizing conditions add small amounts to nitric acid whenever the red-brown NO_2 fumes disappear.	2a. Add nitric acid in 1 ml increments using a measuring pipet.	

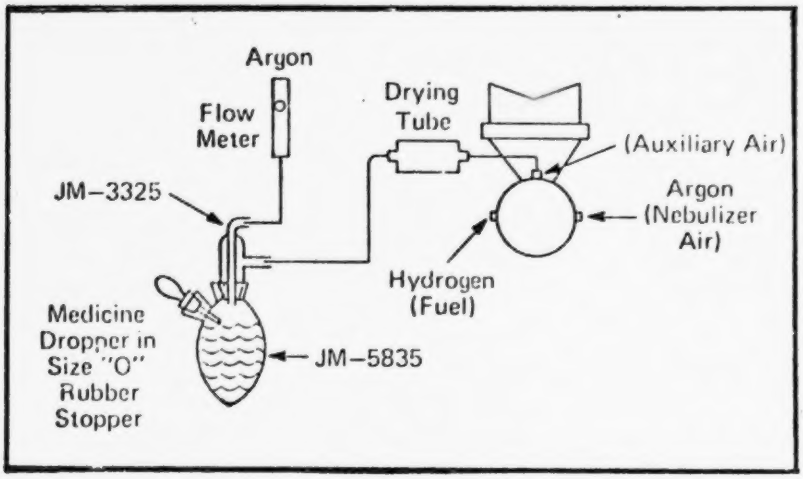
WATER MONITORING PROCEDURE: Determination of Arsenic and Selenium

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Sample Preparation (Continued)	3. Allow to cool slightly, add 25 ml of deionized distilled water and 1 ml perchloric acid, and again evaporate to SO_3 fumes.	3a. Use a 25 ml graduate. Use a 1 ml measuring pipet.	
	4. Allow to cool, add 40 ml concentrated HCL, transfer the solution into a 100 ml volumetric flask and dilute to the mark with deionized distilled water.	4a. Use a plastic wash bottle to rinse the beaker during the transfer. 4b. Use a 50 ml graduate.	
E. Standards Preparation	1. Transfer 0, 0.5, 1.0, 1.5 and 2.0 ml standard arsenic or selenium solution to 100 ml volumetric flasks and bring to volume with diluent to obtain a concentration of 0, 0, 10, 15, and 20 µg/l arsenic or selenium.	1a. Use a 1 ml micro pipet graduated in 0.1 ml. 1b. Refer to reagent preparation section.	
F. Samples and Standards Treatment	1. Transfer a 25 ml aliquot of sample prepared as in (D.1) or D.2) or standard prepared as in (E.) to a reaction vessel.	1a. Use a 25 ml volumetric pipet.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Samples and Standards Treatment (Continued)	2. Add 1 ml of 20 percent potassium iodide to arsenic samples and standards only.	2a. Us a 1 ml measuring pipet. 2b. Omit potassium iodide for selenium determinations.	
	3. Add 0.5 ml of 100 percent stannous chloride solution.	3a. Use a 1 ml measuring pipet. 3b. Allow at least 10 minutes for the arsenic or selenium to be reduced to its lowest oxidation state.	
	4. Attach the reaction vessel to the special gas inlet-outlet glassware.		
	5. Fill the medicine dropper with about 1-1/2 ml of zinc slurry that has been kept in suspension with a magnetic stirrer.		
	6. Firmly insert the stopper containing the medicine dropper into the side neck of the reaction vessel.		
	7. Squeeze the bulb to introduce the zinc slurry into the sample or standard.	7a. The argon carrier gas will sweep the generated metal hydride into the burner. The metal hydride will produce a peak almost immediately.	
	8. When the absorbance reaches its maximum and the recorder pen returns part way to the base line, remove the reaction vessel.		

WATER MONITORING PROCEDURE: Determination of Arsenic and Selenium

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Calculations	1. Draw a standard curve by plotting peak heights of standards verus concentrations of standards.	1a. Use linear graph paper.	
	2. Using your standard curve measure the peak heights of the samples and determine the concentration from the curve.	2a. Take in account any dilution factors. In this case the sample was diluted 1 + 1 with acid, multiply the concentration obtained from the curve by two.	


Argon
Flow Meter
Drying Tube
(Auxiliary Air)
JM-3325
Argon (Nebulizer Air)
Hydrogen (Fuel)
Medicine Dropper in Size "O" Rubber Stopper
JM-5835

Schematic arrangement of equipment for determination of arsenic and selenium.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF NITRATE-NITRITE NITROGEN AND OF NITRATE NITROGEN, CADMIUM REDUCTION METHOD

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

CH.N.n/n.EMP.1a.1.77

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

This operational procedure was developed by:

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EDUCATION AND TECHNICAL BACKGROUND

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M.S. - Chemistry

PhD. - Analytical Biochemistry

1 year Commercial Laboratory Chemist

10 years College Chemistry Instructor

7 years Chemical Consultant to Industry

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EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

1. Objective:

To determine the nitrate-nitrite nitrogen and the nitrate nitrogen content of an effluent.

2. Brief Description of Analysis:

The procedure converts nitrate nitrogen to nitrite nitrogen when the nitrate is passed through a column containing copper-cadmium granules. Nitrate is almost quantitatively reduced to nitrite by this process. The resulting nitrite is determined by reacting the effluent with sulfanilamide and coupling with N - (1-napthyl) - ethylenediamine dihydrochloride to form a highly colored dye which can then be determined colorimetrically. A correction must be made for any nitrite initially present in the sample since the method determines total nitrite. The concentration of nitrite originally present in a sample can be determined by omitting the initial copper-cadmium reduction and carrying out the remainder of the procedure. Separate nitrate-nitrite values for a sample may be obtained by analyzing two aliquots of the same; one with the copper-cadmium reduction step and one without the initial reduction step.

3. Applicability of this Procedure:

   a. Range of Concentration:

   0.01 to 1.0 mg NO_3-NO_2 N/liter
   (The range may be extended for samples by dilution.)

   b. Pretreatment of Samples:

   The Federal Register Guidelines do not specify any pretreatment.

   c. Treatment of Interferences in Samples:

   This procedure includes directions for removal of turbidity and/or of grease and oil from samples. It also includes addition of EDTA to eliminate interferences from metals. No other interferences are noted in the Source of Procedure.*

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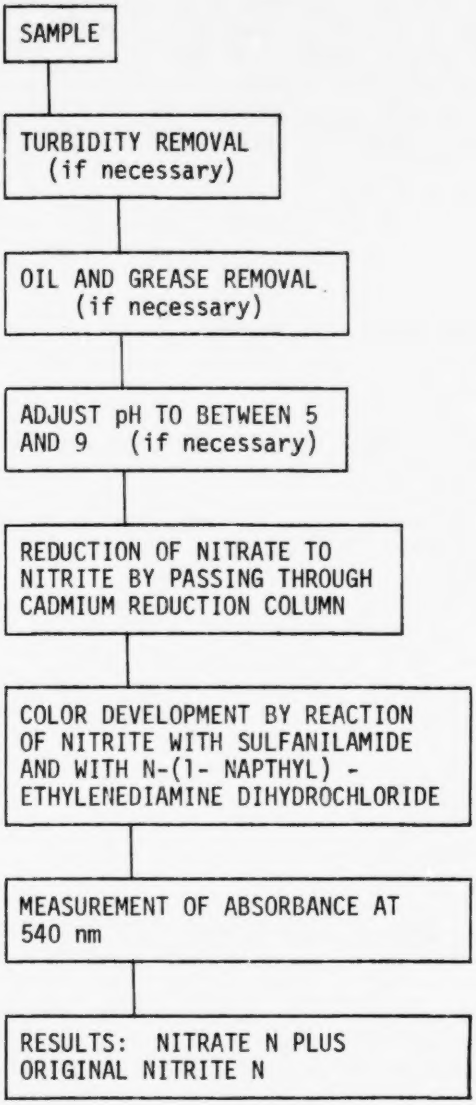
* Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, Cincinnati, Ohio, page 201.

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

FLOW SHEET:


SAMPLE
TURBIDITY REMOVAL (if necessary)
OIL AND GREASE REMOVAL (if necessary)
ADJUST pH TO BETWEEN 5 AND 9 (if necessary)
REDUCTION OF NITRATE TO NITRITE BY PASSING THROUGH CADMIUM REDUCTION COLUMN
COLOR DEVELOPMENT BY REACTION OF NITRITE WITH SULFANILAMIDE AND WITH N-(1- NAPTHYL) - ETHYLENEDIAMINE DIHYDROCHLORIDE
MEASUREMENT OF ABSORBANCE AT 540 nm
RESULTS: NITRATE N PLUS ORIGINAL NITRITE N

The above procedures determine nitrate N plus nitrite N. The initial nitrite concentration of the samples should be determined prior to reduction. Thus, the nitrate concentration can be determined by:

Nitrate N = Total Nitrite N - Nitrite N before reduction

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Balance, analytical, 160 g capacity, precision ± 0.1 mg
2. Balance, triple beam, 500 g capacity, precision ± 0.25 g
3. pH meter/combination electrode, range 0-14 pH
4. Refrigerator, temperature range 2° - 10°C
5. Spectrophotometer, wave length range 325-825 nm
6. Still and de-ionizing cartridges (or other means of distilling and de-ionizing water)

B. Reusable Supplies:

1. One apron, laboratory
2. One 100 ml beaker
3. Four 250 ml beakers (3 for buffer solutions)
4. One 400 ml beaker
5. One 1 liter beaker
6. One 2 liter beaker
7. Two bottles, Barnes with stoppers and two droppers, small gauge
8. One 150 ml bottle, dropper
9. One 250 ml bottle, plastic wash
10. One 100 ml bottle, storage with screw-on cap (storage of 6N HCl)
11. Seven 1 liter bottles, storage, brown with screw-on caps or rubber stoppers
12. Two 5 gallon bottles, water with bottom spout
13. One brush, camel hair (cleaning analytical balance)
14. Two brushes, bottle (cleaning glassware)
15. One bulb, propipet type
16. One buret holder, double clamps (reduction column support)
17. Two columns, reduction (see Figure 1 at the end of this section)
18. Three cuvettes
19. One 25 ml cylinder, graduated
20. One 50 ml cylinder, graduated
21. One 100 ml cylinder, graduated
22. One 500 ml cylinder, graduated
23. One 1 liter cylinder, graduated
24. One 50 ml flask, volumetric with stopper (dilution of sample)
25. Twelve 100 ml flasks, volumetric with stoppers (for standards)
26. X 100 ml flasks, volumetric with stoppers (for samples - 1 flask per sample)
27. Twelve 250 ml flasks, Erlenmeyer with stoppers (for standards)
28. X 250 ml flasks, Erlenmeyer with stoppers (for samples-1 flask per sample)
29. One 1 liter flask, Erlenmeyer, or a large, empty chemical bottle (for Cd washings)
30. Three 1 liter flasks, volumetric with stoppers
31. Two 2 liter flasks, volumetric with stoppers
32. One filter funnel for 0.45 μ filter (turbidity removal)

B. Reusable Supplies (Continued)

33. One funnel, powder
34. One funnel, large powder with large filter paper (for Cd washings)
35. One 250 ml funnel, separatory (oil and grease removal)
36. One pair glasses, safety
37. Two hoses, rubber, 3" strip, 4 cm I.D. with screw type clamp
38. One notebook (recording data)
39. Two 100 ml volumetric pipets (construction of reduction columns)
40. One 0.5 ml pipet, volumetric
41. One 1 ml pipet, volumetric
42. One 2 ml pipet, volumetric
43. One 5 ml pipet, volumetric
44. One 10 ml pipet, volumetric
45. One 25 ml pipet, volumetric
46. One 50 ml pipet, volumetric
47. One rod, stirring (6" or 12")
48. One sieve, 40 mesh
49. One sieve, 60 mesh
50. One spatula (scoopula)
51. Two stands, ring (support funnel, and reduction column)
52. One support, ring, small (support funnel)

C. Consumable Supplies:

1. Glasswool, wad
2. Membrane filter, 0.45 μ
3. Notebook (recording data)
4. Pen or pencil (recording data, marking flasks)
5. Soap
6. Sponges (for cleaning)
7. Tissues, soft (wiping cuvettes and electrodes)
8. Towels, paper
9. Twelve weighing boats
10. 26 g ammonium chloride, NH_4Cl
*11. 100 ml ammonium hydroxide, NH_4OH
*12. 150 ml buffer solution, STD pH 4
*13. 600 ml buffer solution, STD pH 7
*14. 450 ml buffer solution, STD pH 10
**15. 25 g cadmium granules, 40-60 mesh
16. 55 ml chloroform, $CHCl_3$
17. 20 g copper sulfate, pentahydrate, $CuSO_4 \cdot 5H_2O$
18. 3.4 g disodium ethylenediamine tetraacetate, $C_{10}H_{14}N_2Na_2O_8$
19. 1 g N-(1-napthyl) - ethylenediamine dihydrochloride, $C_{12}H_{14}N_2 \cdot 2HCl$
*20. 200 ml hydrochloric acid, concentrated, HCl
21. 100 ml hydrochloric acid, dilute (6N), HCl
22. 100 ml phosphoric acid, concentrated, H_3PO_4
*23. Potassium dichromate (cleaning solution), $K_2Cr_2O_7$
24. 7.218 g potassium nitrate, KNO_3

C. Consumable Supplies (Continued)

25. 6.072 g potassium nitrite, KNO_2
26. 240 g sodium hydroxide, pellets, NaOH
27. 10 g sulfanilamide, $C_6H_8N_2O_2S$
*28. Sulfuric acid, concentrated, (cleaning solution) H_2SO_4
29. 100 g zinc sulfate, heptahydrate, $ZnSO_4 \cdot 7H_2O$
30. Labels, package, 1 1/2 x 1 inch
31. Paper, graph 8 1/2 x 11, package

All reagents should be reagent grade.

The above amounts do not allow for spillage or mistakes.

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*These amounts will vary

**This metal can be purchased from EM Laboratories, Inc., 500 Executive Boulevard, Elmsford, New York,10523, Cat. 2001 cadmium, coarse powder

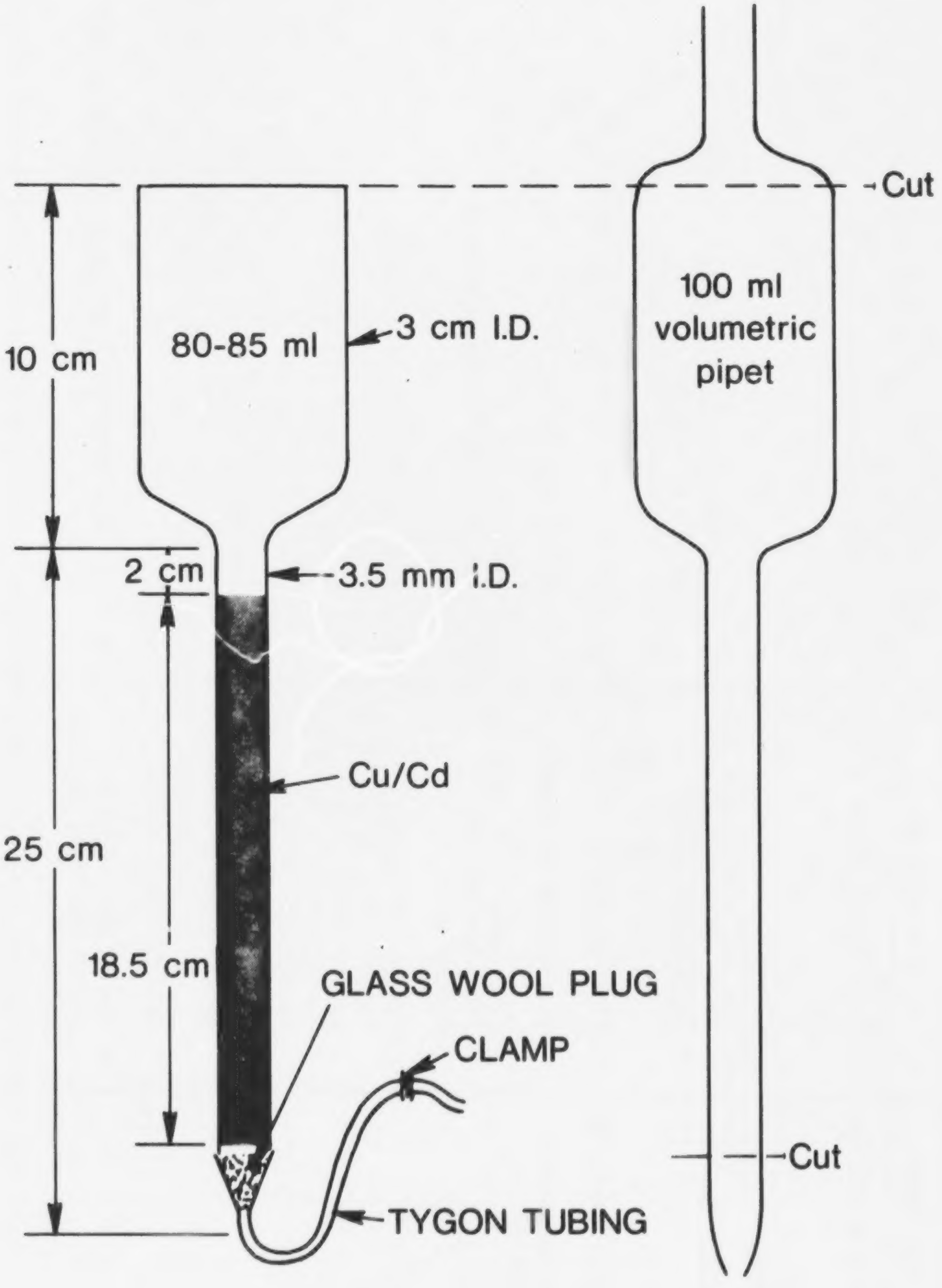
10 cm
80-85 ml
3 cm I.D.
100 ml
volumetric
pipet
Cut
2 cm
3.5 mm I.D.
Cu/Cd
25 cm
18.5 cm
GLASS WOOL PLUG
CLAMP
TYGON TUBING
Cut

Figure 1. Reduction column

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
DETERMINATION OF NITRATE-NITRITE NITROGEN AND OF NITRATE NITROGEN, mg/liter			I (p. 41)
A. Equipment Preparation			
1. Glassware Wash-Up	1. Clean all glassware in suitable detergent.	1a. Distilled water drains without leaving any droplets on surfaces. 1b. Use chromerge if necessary.	
2. Balance Inspection	1. Clean balance.	1a. Free of dust and dirt.	
3. Spectrophotometer Inspection	1. Clean spectrophotometer.	1a. Free of dust and dirt.	
	2. Turn power on by rotating the power control clockwise.	2a. Pilot lamp on. 2b. Directions are for Spectronic 20.	
	3. Select wavelength by rotating the wavelength control knob either direction until the proper wavelength is reached.	3a. 540 nm on the wavelength scale.	
	4. Zero the instrument by bringing the meter needle to "0" on the percent transmittance scale.	4a. Meter needle reads zero.	
	5. Use an empty cell and adjust the light control to 100% T.	5a. To be sure that the instrument can achieve 100% T.	

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and of Nitrate Nitrogen, Cadmium Reduction Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation			
1. Distilled Water	1. Prepare approximately ten (10) liters of highly pure water.	1a. An ion exchange column in conjunction with a still provides an adequate source of highly pure water. 1b. This water will be used for all reagent preparation and washing of equipment. 1c. The pH of the water must be between 5.5-7.5.	
2. Concentrated Ammonium Chloride EDTA Solution	1. Weigh 26 g of ammonium chloride, NH_4Cl, in a weighing boat and wash into 2.0 liter graduated beaker.	1a. Distilled water should be used for all phases of solution preparation including water used in washing a solid into a container.	
	2. Weigh 3.4 g of disodium ethylenediamine tetraacetate, $C_{10}H_{14}N_2Na_2O_8$, and wash into the same beaker.		
	3. Add enough distilled water to bring the total volume to approximately 1800 ml.		
	4. Use a pH meter to adjust the pH of the solution to 8.5 by the dropwise addition of concentrated ammonium hydroxide, NH_4OH.	4a. Mix the solution thoroughly by stirring, after the addition of each drop of NH_4OH.	
	5. After the pH has been adjusted, transfer the solution to a 2 liter volumetric flask.	5a. Whenever a solution is transferred, the container from which the transfer is made should be washed and the washings added to the container to which the transfer was made.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	6. Dilute to volume with distilled water.	6a. The solution is stable for several months.	
	7. Label the bottle in which the solution is stored.	7a. Include the name of the solution, your name and the date of preparation.	
3. Dilute Ammonium Chloride EDTA Solution	1. Measure 1200 ml of the concentrated ammonium chloride-EDTA solution, using a graduated cylinder.		
	2. Pour the measured solution into a 2.0 liter volumetric flask.		
	3. Dilute to volume with distilled water.		
	4. Store in a labeled container.	4a. Both the concentrated and dilute ammonium chloride-EDTA solutions are stable for several months.	
4. Color Reagent	1. Add 800 ml of distilled water to a 1 liter flask.	1a. Use a graduated cylinder. 1b. Use a 1 liter volumetric flask.	
	2. Add 100 ml of concentrated phosphoric acid, H_3PO_4, to the same flask.		
	3. Mix thoroughly.		
	4. Weigh 10 g of sulfanilamide ($C_6H_8N_2O_2S$) in a weighing boat.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	5. Use a wash bottle and funnel to wash the sulfanilamide into the 1 liter flask containing phosphoric acid solution.		
	6. Weigh 1 g N-(1-napthyl)-ethylenediamine dihydrochloride, Marshall's Reagent, and wash into same flask.		
	7. Dilute to volume with distilled water.		
	8. Store in a labeled container.	8a. Container should be dark 1 liter plastic reagent bottle. 8b. Store at 4°C when not in use. 8c. Use at room temperature. 8d. The solution is stable for several months. 8e. A very faint pink color may show up in this color reagent. You may still use the reagent. If a precipitate forms in the reagent, though, discard it.	
5. Zinc Sulfate Solution	1. Weigh 100 g of zinc sulfate heptahydrate, $ZnSO_4 \cdot 7H_2O$, in a weighing boat.	1a. This reagent is used if flocculation is employed as an alternative to filtration if the sample requires removal of turbidity.	
	2. Wash into a 1 liter flask using a wash bottle and a funnel.	2a. Use a volumetric flask.	
	3. Add sufficient distilled water to dissolve all of the solid.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	4. Dilute to volume with distilled water.		
	5. Store in a labeled container.	5a. This solution is stable for at least one year.	
6. Sodium Hydroxide Solution (6N)	1. Rapidly weigh 240 g of solid sodium hydroxide, NaOH, pellets in a 1 liter graduated beaker.	1a. This reagent is used if flocculation is employed as an alternative to filtration if the sample requires removal of turbidity. 1b. Sodium hydroxide picks up moisture from the air quite readily.	
	2. Add 500 ml distilled water to dissolve the sodium hydroxide.	2a. The water should be added with constant swirling to avoid fusing.	
	3. Dilute to a total volume of 1 liter.	3a. The solution should be allowed to cool to room temperature before the dilution is made.	
	4. Store in a glass bottle or jug and stopper with a rubber stopper.	4a. Sodium hydroxide slowly etches glass causing glass stoppers to stick. 4b. The solution is stable for at least a year.	
	5. Label the container.		
7. Ammonium Hydroxide	1. A 100 ml supply should be available.	1a. Drop quantities may be required for pH adjustment.	
	2. Place in a Barnes (dropper) bottle.		
8. Hydrochloric Acid, (6N)	1. Add 50 ml of distilled water to a 400 ml beaker.	1a. A 100 ml graduated cylinder is suitable for measuring the volume of the distilled water and the volume of the acid.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	2. Slowly add 50 ml of concentrated hydrochloric (HCl) acid (12 N) to the same beaker.		
	3. Mix thoroughly.		
	4. Store in a 100 ml bottle.		
	5. Label the container.		
9. Copper Sulfate Solution (2%)	1. Weigh 20 g of copper sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$, in a weighing boat.		
	2. Wash copper sulfate into a one liter volumetric flask.		
	3. Add sufficient distilled water to dissolve the solid.	3a. About 500 ml of water should be sufficient.	
	4. Dilute to volume with distilled water.	4a. This solution is stable for at least one year.	
	5. Store in a labeled container.		
10. Nitrate Stock Solution	1. Carefully weigh 7.218 g of potassium nitrate, KNO_3, in weighing boat.	1a. An analytical balance should be used.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	2. Transfer the solid to a 1 liter volumetric flask equipped with a powder funnel.	2a. This is best achieved by washing the solid onto the funnel with a wash bottle.	
	3. Use wash bottle to wash the solid into the flask.	3a. The weighing boat should be rinsed three times and all of the rinse water should be added to the flask.	
	4. Add sufficient distilled water to dissolve the solid.	4a. About 500 ml is sufficient.	
	5. Dilute to volume with distilled water and thoroughly mix.		
	6. Store in a labeled glass bottle.		
	7. Preserve the solution by adding 2 ml of chloroform, $CHCl_3$.	7a. The solution prepared,stored and preserved in this manner should be stable for at least 6 months. 7b. The nitrate stock solution contains 1.00 mg of nitrate nitrogen (NO_3-N) in each 1.00 ml of solution.	
11. Nitrate Standard Solution	1. Carefully pipet 10.0 ml of nitrate stock solution into a 1 liter volumetric flask.	1a. This nitrate standard solution should be prepared fresh for each use. 1b. The nitrate stock solution should be at room temperature before using. 1c. Use a 10 ml volumetric pipet.	
	2. Dilute to volume with distilled water.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	3. Store in a labeled container.	3a. Use within one hour of preparation. 3b. The nitrate standard solution contains 0.01 mg of nitrate nitrogen (NO_3-N) in each 1.0 ml of solution.	
12. Nitrite Stock Solution	1. Weigh 6.072 g of potassium nitrite, KNO_2, in a weighing boat.	1a. An analytical balance should be used for all weighings involving standards.	
	2. Transfer the solid to a 1 liter volumetric flask using a powder funnel.		
	3. Use wash bottle to wash the solid into the flask.	3a. The weighing boat should be washed three times and the washings added to the flask.	
	4. Add sufficient distilled water to dissolve the solid.	4a. About 500 ml is sufficient.	
	5. Dilute to volume and mix thoroughly.		
	6. Store in a labeled glass bottle.		
	7. Preserve the solution by adding 2 ml of chloroform for each 1 liter of solution and refrigerate when not in use.	7a. The solution should be stable for at least 3 months when preserved this way and stored at about 4°C when not in use. 7b. The nitrite stock solution contains 1.00 mg of nitrite nitrogen (NO_2-N) in each 1.0 ml of solution.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)			
13. Nitrite Standard Solution	1. Pipet 10.0 ml of nitrite stock solution into a 1 liter volumetric flask.	1a. This nitrite standard solution should be prepared fresh for each use. 1b. The nitrite stock solution should be at room temperature before using. 1c. Use a 10 ml volumetric pipet.	
	2. Dilute to volume with distilled water.		
	3. Store in a labeled container.	3a. Use within 1 hour of preparation. 3b. The nitrite standard solution contains 0.01 mg of nitrite nitrogen (NO_2-N) in each 1.0 ml of solution.	
C. Reduction Column Preparation			
1. Preparation of the Glass Column	1. Construct a glass column by joining a 10 cm length of 3 cm ID glass tubing with a 25 cm length of 3.5 mm ID tubing using figure 1 as a guide.	1a. Figure 1 is at the end of the Equipment and Supply Requirements Section. 1b. The column shown in Figure 1 was constructed by cutting both ends off a 100 ml volumetric pipet as indicated. 1c. Fire polish all cut surfaces.	
	2. Loosely plug the delivery tip of the column with glass wool.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)			
2. Preparation of Copperized Cadmium for Packing the Glass Column	1. Weigh about 20 g of cadmium granules in a weighing boat.	1a. This will be enough for one column. 1b. Granulated cadmium (40-60 mesh) can be purchased. 1c. Alternatively, file sticks of pure cadmium metal (reagent grade) with a coarse metal hand file (about second cut) and collect the fraction which passes a sieve with 10 mesh openings and is retained on sieves with 40, then 60 mesh openings. 1d. Handling cadmium is hazardous, thus filing should be conducted under a hood using rubber gloves and mask.	VIII.C.2.1d (p. 46)
	2. Transfer the cadmium to a 400 ml beaker.	2a. A scupula and wash bottle with water is good for this.	
	3. Add enough dilute (6N) hydrochloric acid to cover the granules.		
	4. Swirl the contents of the beaker.		
	5. Pour off the acid while retaining the granules in the beaker.	5a. All decanting should be done into a container equipped with a large funnel and filter paper so as to catch all the small cadmium particles. 5b. Use this filter paper for any subsequent cadmium washings.	
	6. Add enough distilled water to cover the granules.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)	7. Pour off the water while retaining the granules in the beaker.		
	8. Repeat steps 6 and 7, above, two more times so that the granules receive a total of three distilled water washings.		
	9. Add 100 ml of the 2% copper sulfate solution to the granules and swirl for five minutes or until the blue color of the copper sulfate fades.	9a. A brown colloidal (very fine) precipitate of metallic copper may form.	
	10. Carefully decant off the solution leaving the copperized cadmium granules in beaker.	10a. Also decant off through the filter paper any precipitate that formed.	
	11. Repeat steps 9 and 10 until a brown colloidal (very fine) precipitate of metallic copper does form.		
	12. Wash the copper-cadmium at least 10 times with distilled water.	12a. All of the brown precipitated copper should be removed by washing 10 times but continue to wash if any remains.	
	13. Place the washed copper-cadmium on the 60 mesh sieve.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)	14. Pour water over the granules at least three times so that all the small particles will wash through the 60 mesh screen.	14a. Hold the sieve over the filter paper during these washings.	
	15. Return meshed granules to the beaker.	15a. Use a scupula and the wash bottle.	
	16. Decant off excess water used to transfer the cadmium.		
	17. Close the clamp on the column delivery tube.		
	18. Fill the column almost to the top of the cup part with ammonium chloride-EDTA solution.	18a. Use a graduated cylinder and very slowly pour the solution down the inside wall of this column so air pockets do not form.	
	19. Loosely fill the reduction column with copper cadmium granules to a level about 2 cm below the broad, cup-like section as shown in Figure 1.	19a. Avoid tight packing of granules by allowing the granules to "float" down through the solution of ammonium chloride-EDTA. 19b. A glass stirring rod may be used to transfer the cadmium to the column. 19c. For regeneration of column see training guide. 19d. When column is not in use, fill it with ammonium chloride-EDTA solution so that the granules are covered with about 2.5 cm of solution above them.	VII.C.2.19c (p. 43)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reduction Column Preparation (Continued)	20. Open the screw clamp and measure the flow rate of ammonium chloride-EDTA solution through the column.	20a. To calculate the flow rate, place a short 50 ml graduated cylinder under column and measure the amount of fluid collected in one minute. 20b. The flow rate should be between 7 ml and 10 ml/minute. 20c. If the flow rate is too fast, tighten the screw clamp. If the clamp must be so tight that control is lost, add more copper cadmium granules to the column. 20d. If the flow rate is too slow, decrease the length of the copper cadmium column until a flow rate of 7-10 ml/minute is achieved.	
	21. When the flow rate can be maintained between 7 ml and 10 ml/minute, drain off the ammonium chloride-EDTA solution until it is about 2.5 cm above the top of the granules.	21a. When the column is not in use, the copper cadmium granules should be covered with ammonium chloride-EDTA solution so they do not dry out.	
	22. Close the screw clamp.		
D. Removal of Interferences			VI.D (p. 42)
1. Turbidity Removal (If Necessary)	1. Prior to analysis, remove turbidity from samples by filtering through a 0.45 μ membrane filter.	1a. If the turbidity is not removed by filtration, proceed as follows: Add 1 ml of the zinc sulfate solution to 100 ml of sample. Add enough 6 N sodium hydroxide to bring the pH to 10.5, (about 8 to 10 drops is usually sufficient). Let the treated sample stand for 15 minutes. Filter through a 0.45 μ membrane filter. 1b. Suspended solids can clog the reduction column.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Removal of Interferences (Continued)			
2. Oil and Grease Removal (If Necessary)	1. Prior to analysis, measure 100 ml of the sample (filtered sample if the original sample was turbid) into a 400 ml beaker.	1a. Oil and grease can clog the reduction column and coat the Cu/Cd granules.	
	2. By dropwise addition, add sufficient concentrated hydrochloric acid (12 N) to bring the pH down to 2.	2a. Use a pH meter in adjusting the pH to 2. 2b. Standardize using standard buffer of pH = 4.00.	
	3. Place the sample in a 250 ml separatory funnel.		
	4. Add 25 ml of chloroform.		
	5. Shake gently to extract the oils and grease into the chloroform layer.	5a. Carefully release the pressure after shaking gently so that no sample is lost. This can be accomplished by inverting the separatory funnel and slowly opening the stopcock away from face and other people.	
	6. Allow the separatory funnel to stand until all of the chloroform layer settles to the bottom.	6a. Place funnel in ring stand. 6b. Remove stopper while layer is settling.	
	7. Open the stopcock and allow the bottom (chloroform) layer to pass into a 400 ml beaker.	7a. Grease and oils are extracted into chloroform layer leaving a grease-oil free sample which is used for analysis.	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>D. Removal of Interferences (Continued)</td><td>8. Repeat steps 4, 5, 6, and 7 with 25 ml of fresh chloroform.</td><td>8a. The second chloroform extract is added to the same beaker as the first extract.</td><td></td></tr>
<tr><td>E. Preparation of Nitrate Working Standards
1. Nitrate Working Standards</td><td>1. Prepare nitrate working standards by respectively pipetting the following volumes of nitrate standard solution into each of six 100 ml volumetric flasks.
<table>
<tr><th>To Flask No.</th><th>Add This Volume of Nitrate Standard Solution</th><th>For This Concentration of NO_3-N in mg/1</th></tr>
<tr><td>1</td><td>0.0 ml</td><td>0.00</td></tr>
<tr><td>2</td><td>0.5 ml</td><td>0.05</td></tr>
<tr><td>3</td><td>1.0 ml</td><td>0.10</td></tr>
<tr><td>4</td><td>2.0 ml</td><td>0.20</td></tr>
<tr><td>5</td><td>5.0 ml</td><td>0.50</td></tr>
<tr><td>6</td><td>10.0 ml</td><td>1.00</td></tr>
</table>
2. Dilute each of the flasks to volume with distilled water.</td><td>1a. Label flasks.
1b. Use appropriate volumetric pipets (0.5 ml, 1.0 ml, 2.0 ml, 5.0 ml, 10.0 ml).
1c. The 0.00 solution which contains no nitrate (or nitrite) serves as the reagent blank for the nitrate samples and standards which are passed through the reduction column.</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Preparation of Nitrate Working Standards (Continued)	3. Use the working standards immediately after their preparation.		
F. Reduction of Nitrate to Nitrite			
1. Adjustment of pH	1. Use a pH meter to adjust the pH of each of the working standards to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.	1a. Use a beaker small enough for this volume of standard to cover the pH electrode(s). 1b. Make sure that the pH meter is calibrated within this range. 1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter. 1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)	
2. Activation of Column	1. Pipet 25.0 ml of working standard #6 to a small Erlenmeyer flask.	1a. Activation of column is necessary to prepare surfaces of Cu-Cd granules for reduction process. 1b. This standard is 1.00 mg NO_3-N/liter concentration. 1c. A 250 ml flask is good for this purpose.	
	2. Add 75 ml of the dilute ammonium chloride-EDTA solution to the same flask.	2a. A 100 ml graduated cylinder is good for this purpose.	
	3. Mix the working standard thoroughly by swirling the contents of the flask.		
	4. Place a 250 ml beaker under the reduction column.	4a. You will collect the reduced working standard in this beaker.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Reduction of Nitrate to Nitrite (Continued)	5. Check that the level of ammonium chloride-EDTA solution in the column is down to the top of the granules.	5a. If the level is too high, drain the excess into the beaker.	
	6. Pour the prepared nitrate working standard into the reduction column.	6a. Since the column will not hold the total amount, add the final amount after the first 15 ml has passed through the column.	
	7. Using the screw clamp (see Figure 1) adjust the collection rate to 7-10 ml per minute.	7a. The clamp should be slowly opened until a collection rate of 7-10 ml per minute is achieved. 7b. A collection rate of 7-10 ml of solution per minute should be carefully maintained throughout the collection process to assure complete reduction of nitrate in the sample.	
	8. Collect the reduced working standard until the level of solution is 0.5 cm above the top of the granules.		
	9. Close the screw clamp to stop the flow.		
	10. Discard the entire reduced working standard.	10a. The column is now activated.	
	11. Measure about 40 ml of ammonium chloride-EDTA solution.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Reduction of Nitrate to Nitrite (Continued)	12. Pour the 40 ml into the column.		
	13. Repeat steps 8 and 9.	13a. The nitrate standard should now be "washed off" the column.	
3. Reduction of Working Standards	1. Pipet 25.0 ml of the lowest concentration of nitrate working standard into a small Erlenmeyer flask.	1a. A 250 ml flask is good for this purpose. 1b. Label the flask. 1c. Begin with the 0.00 mg/liter solution.	
	2. Add 75 ml of the dilute ammonium chloride-EDTA solution to the same flask.	2a. Use a 100 ml graduated cylinder.	
	3. Mix nitrate working standard thoroughly by swirling the contents of the flask.		
	4. Place a short graduated cylinder under the reduction column.	4a. You need to measure 25 ml of solution in the graduate.	
	5. Pour the prepared nitrate working standard into the reduction column.	5a. Since the column will not hold the total amount, add the final amount after the first 15 ml has passed through the column.	
	6. Using the screw clamp (see Figure 1) adjust the collection rate to 7-10 ml per minute.	6a. The clamp should be slowly opened until a collection rate of 7-10 ml per minute is achieved. 6b. A collection rate of 7-10 ml of solution per minute should be carefully maintained throughout the collection process to assure complete reduction of the nitrate in the nitrate working standard.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Reduction of Nitrate to Nitrite (Continued)	7. Discard the first 25 ml of solution which is collected.	7a. This discard portion serves to "wash off" solution remaining in the column from any previous pass-through.	
	8. Replace the graduate with the rinsed, air-dried flask used for this standard.		
	9. Collect the remaining portion of the reduced standard in the original flask.	9a. Close the screw clamp when the level of solution is about 0.5 cm above the granules. 9b. About 70 ml should be in the flask.	
	10. Analyze the reduced standard immediately after collection from the reduction column.	10a. While one solution is passing through the column you should proceed to color development of the previous solution that has already been reduced. Color development (Section G) must begin within 15 minutes after reduction.	
	11. Repeat steps 1 through 10 for each of the prepared working nitrate standards.	11a. Proceed from the least concentrated to the most concentrated standard. 11b. Label each receiver flask.	
G. Color Development of Reduced Nitrate Working Standards	1. Use a 50.0 ml pipet to remove a 50.0 ml aliquot from flask #1 (0.00 mg/liter NO_3-N).	1a. By using a propipet the aliquot can remain in the pipet during the next two steps. 1b. Aliquots of each of the working standards should have been passed through the reduction column as described in the previous section (Section F). 1c. The reduced working standards should be analyzed as soon as possible after the reduction and in no case should they be allowed to stand for more than 15 minutes after reduction before color development is begun.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Color Development of Reduced Nitrate Working Standards (Continued)	2. Discard the remainder of the nitrate reduced working standard.		
	3. Shake flask dry.	3a. Do not rinse the flask.	
	4. Add the 50.0 ml working standard back to same flask from which it was removed.	4a. If you find the technique in steps 1-4 too difficult, transfer the 50.0 ml to a different flask.	
	5. Add 2.0 ml of the color reagent to the 50.0 ml of working standard.	5a. Use a 2.0 ml volumetric pipet.	
	6. Mix thoroughly by swirling.		
	7. Allow the working standard to stand until color develops.	7a. The reduced working standard should be allowed to stand for at least 10 minutes but not more than two hours before doing Procedure L, Spectrophotometric Measurements.	
	8. Repeat steps 1 through 7 for each of the reduced working standards.	8a. Start with least concentrated solution and proceed to most concentrated. 8b. Rinse the 50.0 ml pipet thoroughly after each standard.	
H. Analysis of Samples for Nitrate Reduced to Nitrite			
1. Dilution of Samples (if necessary)	1. Pipet 25.0 ml of unknown sample into 50 ml volumetric flask.	1a. Potable water samples will usually require no dilution, while sewage samples may require dilution.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Analysis of Samples for Nitrate Reduced to Nitrite (Continued)	2. Dilute to volume with distilled water.	2a. If you need to dilute a sample, you must apply a dilution factor to the concentration found from a standard curve.	VII.H.1.2a (p. 44)
2. Adjustment of pH	1. Use a pH meter to adjust the pH of each sample to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.	1a. Put the 50 ml of sample in a small beaker so the pH electrode(s) is covered with solution. 1b. Make sure that pH meter is calibrated within this range. 1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter. 1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)	
3. Reduction of Nitrate to Nitrite in Samples	1. Aliquots of each of the samples should be passed through the reduction column as described in Procedure F.3, "Reduction of Working Standards."		
4. Color Development in Samples	1. Follow the steps in Procedure G, "Color Development."		
I. Preparation of Nitrite Working Standards			
1. Nitrite Working Standards	1. Prepare nitrite working standards by respectively pipetting the following volumes of nitrite standard solution into each of six 100 ml volumetric flasks.	1a. Label flasks. 1b. Use appropriate volumetric pipets (0.5 ml, 1.0 ml, 2.0 ml, 5.0 ml, 10.0 ml). 1c. The 0.00 solution which contains no nitrite (or nitrate) serves as the reagent blank for the nitrite standards and samples that are not passed through the column.	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>I. Preparation of Nitrite Working Standards (Continued)</td><td>
<table>
<tr><th>To Flask No.</th><th>Add This Volume of Nitrite Standard Solution</th><th>For This Concentration of NO_2-N in mg/l</th></tr>
<tr><td>1</td><td>0.0 ml</td><td>0.00</td></tr>
<tr><td>2</td><td>0.5 ml</td><td>0.05</td></tr>
<tr><td>3</td><td>1.0 ml</td><td>0.10</td></tr>
<tr><td>4</td><td>2.0 ml</td><td>0.20</td></tr>
<tr><td>5</td><td>5.0 ml</td><td>0.50</td></tr>
<tr><td>6</td><td>10.0 ml</td><td>1.00</td></tr>
</table>
2. Dilute each of the flasks to volume with distilled water.
3. Use the working standards immediately after their preparation.</td><td></td><td></td></tr>
<tr><td>2. Adjustment of pH</td><td>1. Use a pH meter to adjust the pH of each of the working standards to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.</td><td>1a. Use a beaker small enough for this volume of standard to cover the pH electrode(s).
1b. Make sure that pH meter is calibrated within this range.
1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter.
1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Color Development of Nitrite Working Standards	1. Pipet 25.0 ml of each of the nitrite working standards into each of six clean 250 ml Erlenmeyer flasks.	1a. Use a 25.0 ml volumetric pipet. 1b. Label each flask. 1c. The nitrite working standards are not passed through the reduction column.	
	2. Add 75 ml of dilute ammonium chloride-EDTA solution to each of the nitrite working standards.	2a. Use a 100 ml graduated cylinder.	
	3. Mix each thoroughly by swirling each flask.		
	4. Use a 50.0 ml pipet to remove a 50.0 ml aliquot from flask #1 (0.00 mg/liter NO_2-N).	4a. By using a propipet the aliquot can remain in the pipet during the next two steps.	
	5. Discard the remainder of the standard from the flask.		
	6. Shake the flask dry.	6a. Do not rinse the flask.	
	7. Add the 50.0 ml nitrite working standard back to the same flask from which it was removed.		
	8. Add 2.0 ml of the color reagent to each nitrite working standard.	8a. Use a 2.0 ml volumetric pipet.	
	9. Mix thoroughly by swirling.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Color Development of Nitrite Working Standards (Continued)	10. Allow the working standards to stand until color develops.	10a. At least 10 minutes but no more than 2 hours should be allowed before doing Procedure L, Spectrophotometric Measurements.	
	11. Repeat steps 4 through 10 for each of the nitrite standards.	11a. Proceed from the least concentrated to the most concentrated standard. 11b. Rinse the 50.0 ml pipet thoroughly after each standard.	
K. Analysis of Non-reduced Samples for Nitrite			
1. Dilution of Samples (if necessary)	1. Pipet 25.0 ml of unknown sample into 50 ml volumetric flask.	1a. NOTE: Potable water samples will usually require no dilution, while sewage samples may require dilution.	
	2. Dilute to volume with distilled water.	2a. If you need to dilute a sample, you must apply a dilution factor to get a final answer.	VII.K.1.2a (p. 44)
2. Adjustment of pH	1. Use a pH meter to adjust the pH of each sample to between 5 and 9 either with concentrated hydrochloric acid or with concentrated ammonium hydroxide.	1a. Put the 50 ml of sample in a small beaker so the pH electrode(s) is covered with solution. 1b. Make sure that pH meter is calibrated within this range. 1c. Use buffer solutions pH 4, pH 7, pH 10 to calibrate and check the meter. 1d. This pH adjustment is necessary to insure that the pH is approximately 8.5 (No pH adjustment is necessary if the pH is already between 5 and 9.)	
3. Color Development	1. Pipet 25.0 ml of sample into a clean 250 ml Erlenmeyer flask.	1a. Use a 25.0 ml volumetric pipet. 1b. Label the flask. 1c. The sample is not passed through the reduction column.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
K. Analysis of Non-reduced Samples for Nitrite (Continued)	2. Add 75 ml of the dilute ammonium chloride-EDTA solution to the same flask.	2a. Use a 100 ml graduated cylinder.	
	3. Mix the sample thoroughly by swirling.		
	4. Use a 50.0 ml pipet to remove a 50.0 ml aliquot from flask.	4a. By using a propipet the aliquot can remain in the pipet during the next two steps.	
	5. Discard the remainder of the solution from the flask.		
	6. Shake flask dry.	6a. Do not rinse the flask.	
	7. Add the 50.0 ml of sample back to same flask from which it was removed.		
	8. Add 2.0 ml of the color reagent to the same flask.	8a. Use a 2.0 ml volumetric pipet.	
	9. Mix the sample thoroughly by swirling.		
	10. Allow the sample to stand until color develops.	10a. At least 10 minutes but no more than 2 hours should be allowed before doing Procedure L, Spectrophotometric Measurements.	
	11. Repeat steps 1 through 10 for each sample.	11a. Rinse the 50.0 ml pipet thoroughly after each sample.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
L. Spectrophotometric Measurements			
1. Adjusting the Instrument	1. Consult the manufacturer's instructions for calibrating your particular instrument.	1a. Instrument must be warmed up for at least 10 minutes. 1b. There is an EMP on "Use of a Spectrophotometer."	
	2. Adjust the wavelength to 540 nm.		
	3. Check to make sure that the instrument reads infinite absorbance with no sample cell in the instrument.	3a. If it does not read infinite absorbance with no sample cell in it, adjust the instrument so that it does read infinite absorbance (see manufacturer's instructions). 3b. Use on and off switch to calibrate infinite absorbance.	
2. Reduced Nitrate Standards and Sample(s)	1. Use the reduced nitrate reagent blank to adjust the instrument to zero absorbance.	1a. Use 0.00 nitrate working standard reagent blank which has been passed through the column. 1b. Adjust to zero absorbance using the calibration knob.	
	2. Measure and record the absorbance of each reduced nitrate working standard.	2a. Use the nitrate working standards which have been passed through the column. 2b. Use data sheet provided.	IX.L.2.2b (p. 47)
	3. Measure and record the absorbance for each reduced sample.	3a. Use data sheet provided.	
3. Non-reduced Nitrite Standards and Sample(s)	1. Use the nitrite reagent blank (non-reduced) to adjust the instrument to zero absorbance.	1a. Use 0.00 nitrite working standard reagent blank. 1b. Adjust to zero absorbance using the calibration knob.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
L. Spectrophotometric Measurements (Continued)	2. Measure and record the absorbance of each non-reduced nitrite working standard.	2a. Use data sheet provided.	IX (pX.L.3.2a p. 47)
	3. Measure and record the absorbance for each non-reduced sample.	3a. Use data sheet provided.	
M. Preparation of Calibration Curve	1. Obtain an 8 1/2 x 11 inch piece of graph paper.		
	2. Label the longer side as the concentration axis.	2a. See Training Guide for an example of labeling the axis on a calibration curve.	VI (pII.M.2a p. 45)
	3. Label the shorter side as the absorbance axis.		
	4. Use the absorbance value and its corresponding nitrate concentration for each of the nitrate working standards to make a plot of absorbance versus concentration.	4a. Use the absorbances and concentrations recorded on the data sheet in Column B, "Total NO_2+NO_3-N." 4b. This will be the standard curve for reduced samples.	IX (pX.M.4a p. 47)
	5. On another piece of graph paper follow steps 1, 2, 3, and 4 using absorbance values and the corresponding nitrite concentrations for each of the nitrite working standards.	5a. Use the absorbances and concentrations recorded on the data sheet in Column D, "NO_2-N." 5b. This will be the standard curve for non-reduced samples.	IX (pX.M.5a p. 47)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
N. Checking Column Efficiency	1. Divide the absorbance value for the 1.00 mg/liter NITRATE (NO_3) working standard by the absorbance for the 1.00 mg/liter NITRITE (NO_2) working standard to obtain the column efficiency as follows:	1a. The abbreviation, abs is used to stand for absorbance.	
	$\frac{\text{abs of 1.00 mg/liter } NO_3 \text{ std}}{\text{abs of 1.00 mg/liter } NO_2 \text{ std}} \times 100 = \%$ efficiency		
	2. Divide the absorbance values for each of the other NITRATE (NO_3) working standards by the absorbance value for the corresponding NITRITE (NO_2) working standard to obtain a column efficiency value in each case as was done in the previous step.		
	3. Calculate the average value for the column efficiency.	3a. The average value for the column efficiency should be between 96% and 104%. If the average % efficiency does not fall in this range, another cadmium reduction column should be prepared and tested until the average column efficiency does fall in this range. 3b. For regeneration of a column, see Training Guide.	VII.N.3b (p. 43)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
O. Determination of mg/liter Nitrite Nitrogen Plus Nitrate Nitrogen in a Sample	1. Use the absorbance for the reduced sample and the standard curve for reduced samples ("Total NO_2+NO_3-N") to obtain the mg/liter of nitrite-N plus nitrate-N in the sample and record it in Column (A) on the data sheet provided.	1a. If the sample was not diluted (25 ml of sample is used), the mg/liter result is read directly from the nitrate standard curve. 1b. If the concentration of nitrate in the sample is too high for analysis, the sample must be diluted. The procedure is described in H.1 and involves diluting the sample to a 50 ml volume. In this case, the mg/liter result from the nitrate standard curve must be multiplied by a dilution factor which would be: $\text{Dilution Factor} = \frac{\text{50 ml}}{\text{ml sample used in dilution}}$ 1c. The reduction process converts the nitrate-N initially present in the sample to nitrite nitrogen and the species analyzed is nitrite nitrogen. 1d. Any nitrite nitrogen initially present in the sample remains as nitrite nitrogen after the reduction. Thus the total nitrite analyzed is the sum of the nitrite initially present and the nitrite which has been formed by reduction of nitrate.	IX.O.1a (p. 47) VII.O.1b (p. 44)
P. Determination of mg/liter Nitrite Nitrogen in a Sample	1. Use the absorbance for the non-reduced sample and the standard curve for non-reduced samples ("NO_2-N") to obtain the mg/liter of nitrite-N in the sample and record it in Column (C) on the data sheet provided.	1a. If the sample was not diluted (25 ml of sample is used), the mg/liter result is read directly from the nitrite standard curve. 1b. If the sample was diluted to a 50 ml volume (as given in H.1), the mg/liter result read from the nitrite standard curve must be multiplied by a dilution factor which would be: $\text{Dilution Factor} = \frac{\text{50 ml}}{\text{ml sample used in dilution}}$	IX.P.1a (p. 47) VII.P.1b (p. 44)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
Q. Calculation of mg/liter Nitrate Nitrogen in a Sample	1. Subtract the mg/liter of nitrite-N in the sample from the mg/liter of nitrite-N plus nitrate-N in the sample to obtain the concentration of nitrate-N. 2. Record the answer in Column (E) on the data sheet provided.	1a. Since the procedure measures the total nitrite concentration in a sample, the nitrite concentration of samples must be determined before reduction and after reduction. The nitrate concentration of a sample is then determined by: $NO_3\text{-N} = (NO_2+NO_3\text{-N})_{\text{TOTAL AFTER REDUCTION}} - (NO_2\text{-N})_{\text{BEFORE REDUCTION}}$ These concentrations were recorded on the data sheet in Columns (A) and (C) respectively.	IX.Q.1a (p. 47)
R. Calculation of mg/liter Nitrate in Sample	1. Multiply the value found for nitrate-nitrogen (NO_3-N) by a factor of 4.43. 2. Record the answer in Column (F) on the data sheet provided.	1a. (NO_3-N) x (4.43) = mg/liter Nitrate in sample. 1b. NO_3-N value was calculated in Procedure Q and recorded in Column (E).	IX.R.1b (p. 47)
S. Calculation of mg/liter Nitrite in Samples	1. Multiply the value found for nitrite-nitrogen (NO_2-N) by a factor of 3.29. 2. Record the answer in Column (G) on the data sheet provided.	1a. (NO_2-N) x (3.29) = mg/liter Nitrite in sample. 1b. NO_2-N value is found by using the calibration curve for non-reduced samples as in Procedure P and recorded in Column (C).	IX.S.1b (p. 47)

TRAINING GUIDE

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Training guide materials are presented here under the headings marked. These standardized headings are used throughout this series of procedures.

FFLUENT MONITORING PROCEDURE: Determination of Nitrate-Nitrite Nitrogen and Nitrate Nitrogen, Cadmium Reduction Method

NTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	The cadmium reduction procedure for nitrate-nitrite nitrogen provides a sensitive method for the determination of nitrate singly, or nitrite and nitrate combined in drinking, surface, and saline waters. The method is commonly used to determine both nitrate-N and nitrite-N in water samples.	
	The procedure described in this EMP is applicable for range of 0.01 to 1.0 mg/liter of nitrate-nitrite nitrogen. However, the range may be extended by appropriate sample dilution.	1. Methods for Chemical Analysis of Water and Wastes, 1974, EPA-MDQARL, Cincinnati, Ohio 45268, p. 201.
	The test described in this instruction can be found in the 1974 EPA Methods Manual on page 201, entitled Nitrogen, Nitrate-Nitrite (Cadmium Reduction Method). Another reference which contains an acceptable procedure for this test is on page 423 of the 14th edition of Standard Methods.	2. Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, New York, p. 423.
	The major sources of nitrogen entering the environment are: through the heavy application of nitrogenous fertilizers which cause agricultural runoffs, as the end products of aerobic stabilization of organic nitrogen, in domestic sewage, through animal and plant processing wastes, in animal manure, through the atmosphere and in various types of industrial effluents.	3. Federal Water Pollution Control Administration Water Quality Criteria, U.S. Government Printing Office, Washington, D.C. 1968.
	While nitrogen is essential to our survival (as in the make-up of amino acids and proteins), when it exists as nitrate and nitrite it can be toxic. A limit of 10 mg/l nitrate-N and 1 mg/l nitrite-N is recommended for public water sources. The desirable criteria is virtually 0 mg/liter.	
	In ruminant animals (i.e. cows) nitrates may be internally reduced by bacteria present in the rumen to nitrites. The nitrites have been found to be toxic to these animals. Dr. Joptha E. Campbell, (Chief, Food Chemistry Unit, Milk and Food Research, Environmental Sanitation Program, Public Health Service, U.S. Department of H.E.W., Cincinnati, Ohio, 1968) has reported methemoglobinemia in cattle receiving water containing 2.790 mg/liter of nitrate.	
	Nitrates in high concentrations have also been found to stimulate vegetative growth under favorable conditions. Heavy undesirable growth in fresh water can lead to eutrification of important waterways.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.	Samples should be analyzed for nitrate nitrogen as soon as possible after sampling to avoid any change in nitrogen balance due to biological activity. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. Samples should be preserved with sulfuric acid if they are to be held more than 24 hours. To preserve samples for analysis, add 2.0 ml of concentrated sulfuric acid per liter of sample and store at 4°C.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.2.19c N.3b	Check the column efficiency when it is suspected that column efficiency is decreasing, as indicated by suspected low concentration levels. Prepare working standard nitrate solutions, and pass them through the column. (Begin at E. Preparation of Nitrate Working Standards.) If the absorbance for the known concentration does not give an average between 96% and 104% of your standard curve value for reduced nitrate standards of equivalent concentration, the column must be reactivated.	

REACTIVATION OF COLUMN

1. Empty cadmium granules from column into a clean beaker.
2. Wash with distilled water 3 times
3. Add enough dilute HCl to cover granules.
4. Swirl contents.
5. Decant HCl.
6. Wash with distilled water 3 times.
7. Add 100 ml $CuSO_4$ solution to granules.
8. Swirl contents of beaker for approximately 5 minutes until the blue color fades to colorless.
9. Decant liquid leaving the granules.
10. Repeat steps 7, 8, and 9 until a very fine brown-red precipitate forms.
11. Wash granules with distilled water (approximately 10 times) until precipitate is removed.
12. Place granules on the 60 mesh sieve.
13. Shake to remove the small particles (the particles which remain on the sieve are the ones you want.)
14. Repack column (packing must be loose).
15. Standard curve using nitrate working standards must be re-established.
16. Check column efficiency as described in N, Checking Column Efficiency.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
H.1.2a K.1.2a O.1b P.1b		

Since a dilution is only part sample, when the absorbance reading obtained for it is converted to a concentration using a calibration curve, the concentration obtained is only that of the dilution. To obtain the mg/liter concentration of the sample, the mg/liter concentration of the dilution must be multiplied times the amount of dilution (must be multiplied times the dilution factor). For a 1/2 dilution (25 ml sample/50 ml total volume) the dilution factor would be 2 (the dilution is only half sample). For a 1/5 dilution (10 ml of sample/50 ml total volume) the dilution factor would be 5. Below is a table of some dilution factors when the sample is diluted to a 50 ml volume.

ml of Sample per 50 ml Total Volume	Amount of Dilution	Dilution Factor
25	1/2	2
10	1/5	5
5	1/10	10
1	1/50	50
0.5	1/100	100
0.05	1/1000	1000

The dilution factor for any dilution may be calculated by dividing the ml of sample used in the dilution into 50:

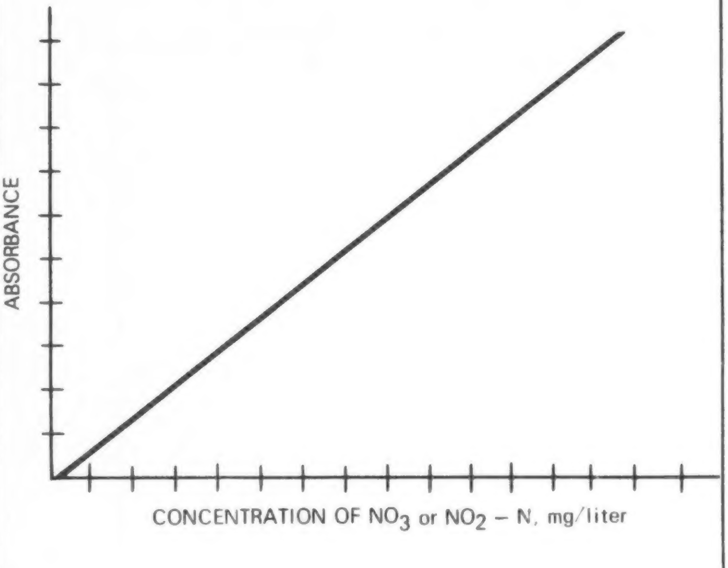
$$\text{Dilution Factor} = \frac{50\ \text{ml}}{\text{ml sample used in dilution}}$$

Ex. 2 ml of sample diluted to 50 ml

$$\frac{50}{2} = 25$$

The dilution factor for this dilution would be 25.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
M.2a	A calibration curve is prepared by plotting the measured absorbance of each of the working standard versus the concentration in the working standard as shown below.	



	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.2.1d	Cadmium metal is highly toxic thus caution must be exercised in the use of cadmium. Cadmium metal should never be handled directly since cadmium has been shown to have cumulative effects. Rubber gloves should be used whenever cadmium must be handled. A mask should be worn during the filing of cadmium and the filing should be done in a hood. The waste cadmium should be disposed of in an appropriate manner which conforms to Federal, State and local pollution control regulations.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	You will need the following Key to use the Example Data Sheet found on the next page:	
	KEY TO DATA SHEET	
L.2.2b M.4a	(B) Record the absorbances of the column-reduced nitrate working standards and of the column-reduced sample(s) in Column (B).	
L.3.2a M.5a	(D) Record the absorbances of the non-reduced nitrite working standards and of the non-reduced sample(s) in Column (D).	
O.1a	(A) Read the mg/liter (concentration) of Total NO_2+NO_3-N in the column-reduced sample(s) from the corresponding calibration curve and record the answer(s) in Column (A).	
P.1a	(C) Read the mg/liter (concentration) of NO_2-N in the non-reduced sample(s) from the corresponding calibration curve and record the answer(s) in Column (C).	
Q.1a	(E) Subtract: Value (A) - Value (C) = Value (E)	
R.1b	(F) Multiply: Value (E) x 4.43 = Value (F)	
S.1b	(G) Multiply: Value (C) x 3.29 = Value (G)	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF NITRATE IN DRINKING WATER AND WASTEWATERS BY THE BRUCINE METHOD

as applied in

WATER TREATMENT FACILITIES
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.N.n/n.lab.WMP.1.11.77

Effluent Monitoring Procedure: Determination of Nitrate in Drinking Water and Wastewaters by the Brucine Method

1. Analysis Objectives;

   The learner will determine the nitrate content of a sample of drinking water or wastewater effluent.

2. Brief Description of Analysis:

   Brucine sulfate-sulfanilic acid color reagent is added to a series of nitrate standards and to the sample. The yellow color which develops is read in a spectrophotometer at 410 nm. A calibration graph is prepared, and the nitrate nitrogen content of the sample is determined from the graph.

3. Applicability of the Procedure:

   The method works well in waters having salinities which range from that of fresh water to sea water.

   a. Range of Concentration:

      The method is recommended for use in the range of 0.1-2.0 mg of nitrate nitrogen/l.

   b. Pretreatment of Sample:

      Filtration through a 0.45 μm membrane in the case of samples which are turbid or contain solids. See C.2.

   c. Treatment of Interferences in the Sample:

      Chlorine is an interference and is removed by addition of sodium arsenite solution. See C.e.

4. Source of Procedure:

   Standard Methods for the Examination of Water and Wastewater, 14th ed., pg. 461, Method 213C, 1975.

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EFFLUENT MONITORING PROCEDURE: Determination of Nitrate in Drinking Water and Wastewaters by the Brucine Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation 1. Cleaning of glassware	1. Clean all Glassware and rinse with tap and distilled water.		V.A.1.1. (p. 16)
2. Balance Inspection	1. Check all balances for cleanliness and proper operation.	1a. Consult the manufacturer's manual if the balance does not operate properly.	
B. Reagent Preparation 1. Distilled Water	1. Prepare approximately five liters of distilled water for use in this procedure.	1a. Either distill the water, or obtain distilled water from some other source.	
		1b. Throughout the remainder of this procedure, unless otherwise stated, the term water means distilled water.	
2. Stock Nitrate Solution	1. Add about 500 ml of water to a 1 liter volumetric flask.	1a. Estimate the 500 ml.	
	2. Weigh 0.7218 g of anhydrous potassium nitrate	2a. Use an analytical balance.	
	3. Transfer it to the flask.		
	4. Swirl the flask.	4a. To dissolve the solid.	
	5. Add water to the 1000 ml mark.		
	6. Thoroughly mix the contents of the flask.	6a. The concentration of this solution is 0.1 mg N/ml.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	7. Transfer the solution to a 1 liter glass stoppered bottle.		
3. Standard Nitrate Solution	1. Pipet 10.0 ml of the stock nitrate solution into a 1 liter volumetric flask.	1a. Prepare this solution just prior to use. 1b. Use a volumetric pipet.	
	2. Add water to the 1000 ml mark.	2a. Use a trip balance	
	3. Thoroughly mix the contents of the flask.	3a. The concentration of this solution is 1.0 µg N/ml.	
	4. Transfer the solution to a 1 liter glass stoppered bottle.		
4. Brucine Sulfate-Sulfanilic acid Solution	1. Weigh 1.0 g of brucine sulfate.	1a. Caution: this material is extremely toxic. If any is spilled, wipe it up with damp tissues, discard the tissues, and wash your hands thoroughly. 1b. Use a trip balance.	
	2. Weigh 0.1 g of sulfanilic acid.		
	3. Measure 70 ml of water.	3a. Use a 100 ml graduated cylinder.	
	4. Transfer it to a 250 ml Erlenmeyer flask.		
	5. Heat the water to almost boiling.		
	6. Transfer the brucine sulfate and sulfanilic acid to the flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	7. Measure 3 ml of concentrated hydrochloric acid, HCl.	7a. Use a 10 ml graduated cylinder.	
	8. Add it to the flask.		
	9. Swirl the flask.	9a. To dissolve the solids.	
	10. Allow the solution to cool to room temperature.		
	11. Measure 30 ml of water.	11a. Use a 100 ml graduated cylinder.	
	12. Transfer it to the flask.		
	13. Thoroughly mix the contents of the flask.	13a. Caution: this solution is also extremely toxic.	
	14. Transfer the solution to a 100 ml glass stoppered bottle.		
5. Sulfuric acid Solution	1. Measure 125 ml of water.	1a. Use a 100 ml graduated cylinder.	
	2. Transfer it to a 1 liter Erlenmeyer flask.		
	3. Measure 500 ml of concentrated sulfuric acid, H_2SO_4.	3a. Use a 500 ml graduated cylinder.	
	4. Pour about 100 ml of the acid into the Erlenmeyer flask.	4a. Pour it down the sides of the flask.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	5. Thoroughly mix the contents of the flask.	5a. Caution: a large amount of heat will be generated.	
	6. Repeat steps 4 and 5 until all of the acid has been added.		
	7. Allow the solution to cool to room temperature.		
	8. Transfer the solution to a 1 liter glass stoppered bottle.		
6. Sodium Chloride Solution	1. Add about 70 ml of water.	1a. Prepare this solution only if the sample is known to contain chloride. Two ml of it are needed per sample and standard. 1b. Estimate the 70 ml.	
	2. Transfer it to a 250 ml Erlenmeyer flask which has a mark at the 100 ml level.		
	3. Weigh 30 g of sodium chloride, NaCl.		
	4. Add it to the flask.		
	5. Swirl the flask.	5a. To dissolve the solid.	
	6. Add water to the 100 ml mark.		
	7. Thoroughly mix the contents of the flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	8. Transfer the solution to a 100 ml glass stoppered bottle.		
7. Sodium Arsenite Solution	1. Add about 50 ml of water to a 100 ml volumetric flask.	1a. Prepare this solution only if the sample is known to contain chlorine. 1b. Estimate the 50 ml.	
	2. Weigh 0.5 g of sodium arsenite, $NaAsO_2$.	2a. Caution: this material is extremely toxic. If any is spilled, wipe it up with damp tissues, discard the tissues, and wash your hands thoroughly. 2b. Use a trip balance.	
	3. Transfer it to the volumetric flask.		
	4. Swirl the flask.	4a. To dissolve the soid.	
	5. Add water to the 100 ml mark.		
	6. Thoroughly mix the contents of the flask.	6a. Caution: This solution is also extremely toxic.	
	7. Transfer it to a 100 ml glass stoppered bottle.		
C. Sample Pretreatment 1. Storage and Preservation	1. Start the nitrate determination promptly after sampling.	1a. Standard Methods offers three preservation techniques when needed: i) Concentrated sulfuric acid, 0.8 ml/liter of sample. If this technique is used, the sample must be neutralized to a pH of about 7 just	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Pretreatment (Continued)		before beginning the determination. ii) Mercuric chloride, 54 mg/liter of sample. iii) Chilling to just above the freezing point. 1b. For later convenience in handling the chlorine interference, filter 1 liter of sample.	
2. Solids, Turbidity	1. If solids and/or turbidity are present in the sample, filter it through a 0.45 μm membrane filter.	1a. The filter will be plugged quickly by solids in the sample. However, only about 20 ml of filtrate is needed for the determination.	
3. Chlorine	1. Determine the free and combined chlorine content of the sample in mg/l.	1a. For samples of wastewater effluent, two methods are approved: amperometric or titrimetric. For samples of drinking water, one method is approved: N, N- Diethyl-Phenylenediamine (DPD). See the appropriate Effluent Monitoring Procedures (EMP's).	
	2. For each 0.1 mg of chlorine (free and combined), add 1 drop of the sodium arsenite solution.	2a. Example calculation: 0.1 = mg of free chlorine per liter of sample 1.0 = mg of combined chlorine per liter of sample Then 1.1 = mg of free and combined chlorine per liter of sample. Therefore add 11 drops of the sodium arsenite for each 1 liter of sample. Only about 10 ml of sample will actually be needed for the determination, but it is convenient to actually collect a larger volume such as 1 liter.	
	3. For each 50 ml of sample, add 1 drop of the sodium arsenite in excess.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Pretreatment (Continued)	4. Thoroughly mix the contents of the sample container.		
D. Procedure 1. Sample	1. Assemble 9 large test tubes in a wire rack.	1a. There must be an empty rack space next to each tube.	
	2. Pipet 1.0 ml of sample into a large test tube.	2a. Use a graduated pipet.	
	3. Pipet 9.0 ml of water into the same tube.	3a. Use a graduated pipet.	
	4. Mix the contents of the tube.	4a. This is a 10% sample dilution.	
	5. Pipet 5.0 ml of the same sample into a second large test tube.	5a. Use the same pipet as in 2a.	
	6. Pipet 5.0 ml of water into the same tube.	6a. Use the same pipet as in 3a.	
	7. Mix the contents of the tube.	7a. This is a 50% sample dilution.	
	8. Pipet 10.0 ml of the same sample into a third large test tube.	8a. Use the same pipet as in 2a. 8b. This is an undiluted, or 100%, sample. 8c. Use a clean graduated pipet to measure volumes of different samples. 8d. The idea of preparing three sample dilutions is that one of them will give a result within the applicable range of the test. Once experience with the sample source is gained, it will not be necessary to do more than one sample dilution.	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>D. Procedure (Continued)
2. Standards</td><td>1. Pipet standard nitrate solution (B.3) and water into the six remaining large test tubes.</td><td>1a. So as to obtain the following solutions:
<table>
<tr><th>Tube Number</th><th>ml of Standard Nitrate Solution</th><th>ml of Water</th><th>Conc. of Nitrate Nitrogen in mg/l</th></tr>
<tr><td>1</td><td>0.0</td><td>10.0</td><td>0.0</td></tr>
<tr><td>2</td><td>1.0</td><td>9.0</td><td>0.1</td></tr>
<tr><td>3</td><td>2.0</td><td>8.0</td><td>0.2</td></tr>
<tr><td>4</td><td>4.0</td><td>6.0</td><td>0.4</td></tr>
<tr><td>5</td><td>7.0</td><td>3.0</td><td>0.7</td></tr>
<tr><td>6</td><td>10.0</td><td>0.0</td><td>1.0</td></tr>
</table>
1b. Use one 10 ml graduated pipet for the standard nitrate, and a second for the water.</td><td></td></tr>
<tr><td>3. Sodium Chloride</td><td>1. Place the rack in a tray containing tap water or cold water from a fountain.</td><td>1a. The water level should be about half-way up the tubes.</td><td></td></tr>
<tr><td></td><td>2. Add about 6 ice cubes to the tray.</td><td>2a. If cold water is used, step 2 is unnecessary.</td><td></td></tr>
<tr><td></td><td>3. Pipet 2.0 ml of the sodium chloride solution into each of the 9 tubes.</td><td>3a. Use a 10 ml graduated pipet only if the sample contains Cl^-.</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (Continued)	4. Thoroughly mix the contents of each of the 9 tubes.	4a. By hand swirling. 4b. Do not use a vortex-type mixer.	
4. Sulfuric Acid	1. Pipet 10.0 ml of the sulfuric acid solution into each of the 9 tubes.	1a. The rack is still in the cold water. 1b. Use a 10 ml graduated pipet.	
	2. Thoroughly mix the contents of each of the 9 tubes.	2a. By hand swirling. 2b. Do not use a vortex-type mixer.	
	3. Allow the tube contents to cool to room temperature.	3a. The ice cubes or cold water will probably have to be replaced. 3b. Do this step only if the sample is known to contain chloride.	
5. Turbidity	1. Check the three sample tubes for turbidity.	1a. If more than one type of sample is being run, there may be more than three sample tubes. Check all the sample tubes for turbidity. 1b. If no turbidity is present, proceed to D.6, Color Development. 1c. If turbidity is present, do steps 2, 3, and 4 below.	
	2. Turn on the Spec 20 and allow it to warm up.		
	3. "Zero" the instrument using tube number 1.	3a. At 410 μm.	
	4. Measure and record the absorbancies of those sample tubes whose contents are turbid.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (Continued) 6. Color Development	1. Pipet 0.5 ml of the brucine sulfate-sulfanilic acid reagent into each of the 9 tubes.	1a. The rack is still in the cold water. 1b. Use a 10 ml graduated pipet.	
	2. Thoroughly mix the contents of each of the 9 tubes.	2a. By hand swirling. 2b. Do not use a vortex-type mixer.	
	3. Place the rack of tubes in the hot water bath.	3a. Ninety-five degrees C.	
	4. If the Spec 20 has not been used prior to this step, turn it on now and allow it to warm up.		
	5. After exactly 20 minutes remove the rack from the hot water and place it back into the cold water.	5a. The ice cubes or cold water may have to be replaced. 5b. The contents of the tubes should be at room temperature.	
7. Color Measurement	1. Remove the rack from the cold water bath.		
	2. Dry the outside of the large test tubes.		
	3. Zero the Spec 20 using tube 1 as a "blank."		
	4. Record the absorbanices for tubes 2 through 6.	4a. These six tubes are the standards to be used in preparing the calibration graph.	
	5. Record the absorbancies for all sample tubes.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTE
E. Calculations	1. Prepare a calibration graph using the absorbancies and concentrations for tubes 1 through 6. 2. If any absorbancies due to turbidity were recorded for any of the sample tubes, subtract those absorbancies from the appropriate absorbancies obtained in D.7.5 above. 3. Determine the mg of nitrate nitrogen per liter of sample for each different sample.		

EFFLUENT MONITORING PROCEDURE: Determination of Nitrate in Drinking Water and Wastewaters by the Brucine Method

TRAINING GUIDE

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II	Educational Concepts - Mathematics
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IX	Records and Reports

Training Guide materials are presented here under the headings marked. These standardized headings are used through this series of proceudres.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required.	
	1. Pour 35 ml of distilled water in a 250 ml beaker.	14th Standard Methods, p. 336, section 2.c.2)
	2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dichromate, $Na_2Cr_2O_7$, to the water.	
	3. Swirl the beaker until the sodium dichromate has dissolved.	
	4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve.	
	5. Pour the solution into a 2 liter beaker.	
	6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker. Caution: Use eyeglasses and protective clothing.	
	7. Stir the mixture thoroughly.	
	8. Store it in a glass stoppered bottle.	
	9. The cleaning solution should be at a temperature of about 50°C when it is used.	
	10. It may therefore be necessary to warm the cleaning solution.	
	11. When using the warm cleaning solution, fill the piece of glassware with the solution.	
	12. Allow it to soak for 2-3 minutes (or longer).	
	13. Pour the cleaning solution back into the storage bottle.	
	14. Rinse the piece of glassware ten times with tap water.	
	15. The cleaning solution may be reused until it turns green.	
	16. It should then be discarded.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF FLUORIDE IN POTABLE AND WASTEWATERS USING THE SPADNS COLORIMETRIC PROCEDURE

as applied in

WATER TREATMENT FACILITIES
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Permits and Operations Division
Office of Water Program Operations
U.S. Environmental Protection Agency

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EFFLUENT MONITORING PROCEDURE: Determination of Fluoride in Potable and Waste Waters Using the SPADNS Colorimetric Procedure

1. Analysis Objectives:

The learner will determine the fluoride content of a water or wastewater sample using the SPADNS Colorimetric Procedure.

2. Brief Description of Analysis:

After distillation to remove interferences the sample is treated with the SPADNS reagent. The amount of color remaining after the reaction of the fluoride and reagents is read on a spectrophotometer. This reading is a function of the fluoride concentration.

3. Applicability of this procedure:

a. Range of Concentration: from 0.05 to 1.4 mg F liter.
b. Pretreatment of Sample:

The Bellack distillation procedure must be carried out on all samples before determination by the SPADNS procedure. The method is applicable to the measurement of fluoride in drinking, surface, and saline waters, domestic and industrial wastes. The distillation procedure is covered in a separate EMP.

c. Treatment of Interferences in the Sample:

The distillation will remove all interferences when carried out properly. For a list of interferences consult the training guide notes (VII.B.2.a).

4. Source of Procedure:

Standard Methods for the Examination of Water and Wastewater; 14th Edition 1975. pg 393.

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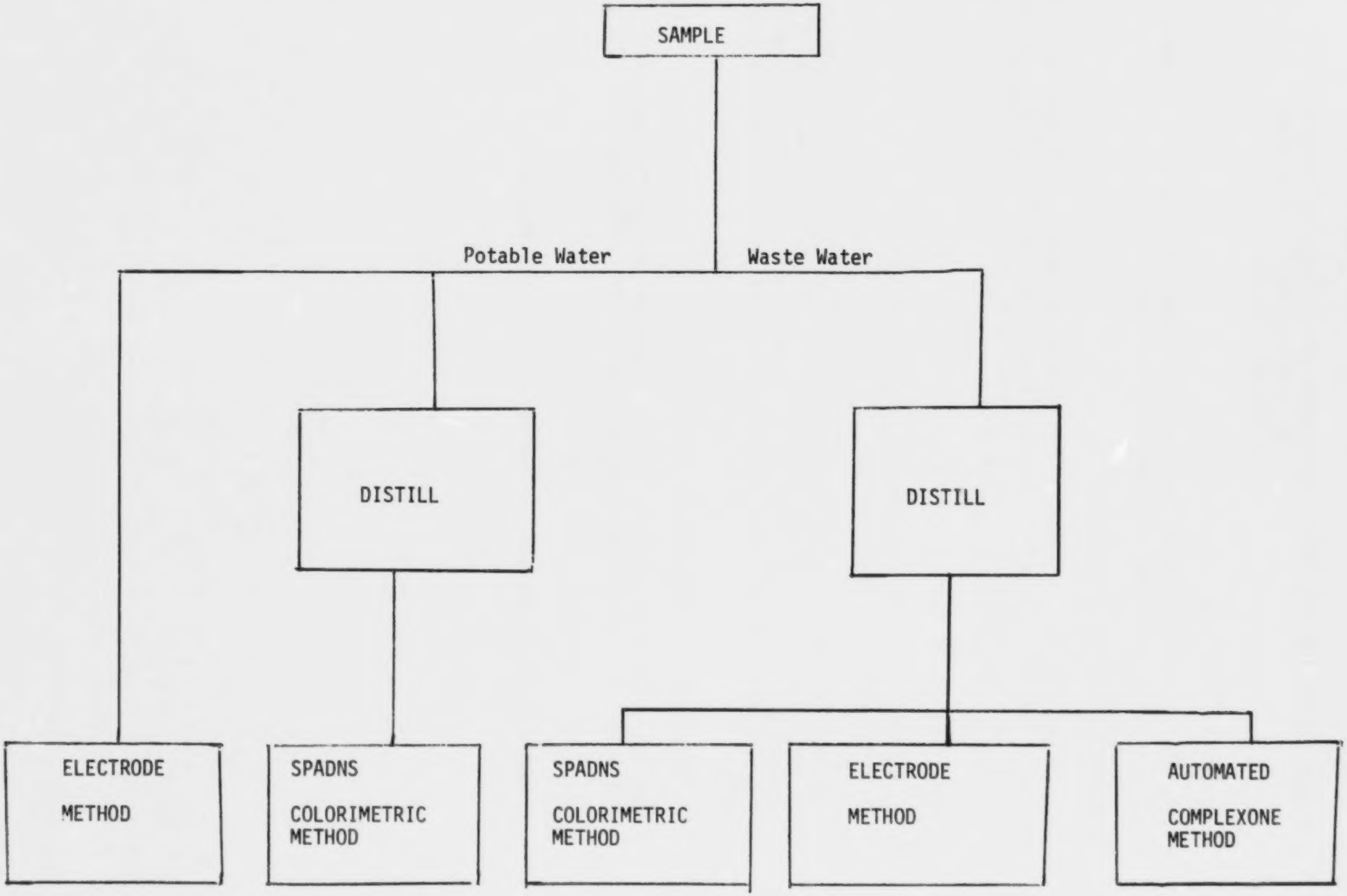
FLUORIDE ANALYSIS FLOW SHEET


SAMPLE
Potable Water
Waste Water
DISTILL
DISTILL
ELECTRODE METHOD
SPADNS COLORIMETRIC METHOD
SPADNS COLORIMETRIC METHOD
ELECTRODE METHOD
AUTOMATED COMPLEXONE METHOD

EFFLUENT MONITORING PROCEDURE: Determination of Fluoride in Potable and Waste Waters Using the SPADNS Colorimetric Procedure

Equipment and Supply Requirements

A. Capital Equipment:

1. Spectrophotometer, for use at 570 nm providing a light path of at least 1 cm. with cells.
2. Analytical balance; capable of weighing to 0.1 mg
3. Still or Ion Exchange column or other source of distilled water
4. Still for distilling sample - see EMP on Fluoride Distillation
5. Trip balance - 500 gram capacity

B. Reusable Supplies:

1. Beakers - 500 ml glass - 1 each
2. Flasks - Erlenmeyer - 125 ml - 8 each
3. Flasks - Erlenmeyer - 500 ml - 1 each
4. Flasks, volumetric - 50 ml - 8 each
5. Flasks, volumetric 100 ml - 2 each
6. Flasks, volumetric 1000 ml - 2 each
7. Graduated Cylinder - 500 ml - 1 each
8. Pipets, volumetric - 10 ml - 3 each
9. Pipets, volumetric - 50 ml - 8 each
10. Powder funnel - 1 each
11. Saftey glasses - 1 pair
12. Spatula - 1 each
13. Thermometer 0-100°C - 1 each
14. Wash bottle - plastic - 1 each

C. Consumable Supplies

1. SPADNS - Reagent 4,5-dihydroxy-3 [(p-sulfophenyl)azo-2.7 napthalene disulfonic acid, trisodium salt.

   Baker Cat No. 5189 --- 10 grams

   Eastman Cat No. 7309 --- 25 grams

2. Zirconyl chloride - Reagent $ZrOCl_2$

   Baker Cat No. X720 --- 500 grams

   Fisher Cat No. Z-80 --- 1 lb.

3. Sodium Fluoride-NaF-Reagent

   Baker Cat No. 3688 --- 1 lb.

   Fisher Cat No. 5299 --- 1/4 lb.

   or

   Sodium Fluoride Stock Solution

C. Consumable Supplies (continued)

3. Orion Research Inc. Cat No. 94-06-07

   Hach Chemical Co.

4. Sodium Arsenite $NaAsO_2$ Reagent

   Baker Cat No. 3487 --- 1 lb.

   Fisher Cat No. S-225 --- 1 lb.

5. Hydrochloric acid HCl Reagent

   Baker Cat No. 9535 --- 1 pt

   Fisher Cat No. A-144 --- 1 pt

6. Weighing boats-plastic disposable - 60 each
7. Pen or Pencil
8. Notebook

D. Addresses of Suppliers Mentioned

J. T. Baker, Chemical Co.
1 Public Square
Cleveland OH 44113

Eastman Organic Chemicals
Eastman Kodak Co.
1187 Ridge Road W.
Rochester, NY 14650

Fisher Scientic Co.
5481 Creek Rd.
Cincinnati, OH 45242

Hach Chemical Co.
P. O. Box 907
Ames, Iowa 50010

Orion Research Inc.
380 Putnam Ave.
Cambridge, MA 02139

EFFLUENT MONITORING PROCEDURE: Determination of Fluoride Using the SPADNS Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Sample Collection	1. Collect a minimum of 300 ml in a plastic or hard glass container.	1a. Do not rinse the container in tap water for its final rinse. Tap water usually contains some fluoride even if the source is not fluoridating. 1b. For distillation a volume of 300 ml is required. For the SPADNS procedure 50 ml or the distillate is used. 1c. No special requirements are necessary for preservation. 1d. Polyethylene bottles are preferred, hard glass (Pyrex, Kimax) is acceptable	
B. Sample pretreatment	1. Add 1 drop (0.05ml) sodium arsenite ($NaAsO_2$) solution for every 0.1 mg residual Cl.	1a. Chlorine will bleach the color and is therefore a definite interference. 1b. Caution: This solution (Reagent D.6.) is toxic. Take care not to injest any.	
	2. Distill the sample.	2a. For total or total dissolved Fluoride the sample must be distilled. 2b. If interferences are present the sample must be distilled. 2c. The distillation procedure is covered under a separate EMP.	I.B.2 (p. 18) VII B.2a (p. 20)
C. Equipment Preparation			
1. Glassware	1. Rinse all glassware immediately after use with tapwater.	1a. If detergent is used care should be taken to rinse thoroughly to remove any phosphate.	
	2. Rinse several times with distilled water.	2a. All tap water contains traces of Fluoride and could also contain about 1.0mg/l if fluoridated.	
2. Spectrophotometer Inspection	1. Clean spectrophotometer.	1a. Free of dust and dirt. 1b. Consult manufacturer's instructions.	

EFFLUENT MONITORING PROCEDURE: Determination of Fluoride Using the SPADNS Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Reagent Preparation			
1. Distilled Water	1. Prepare about six liters of distilled water. This water should be free from fluoride.	1a. Use a still or pass tap water through an ion-exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin.	
2. SPADNS Reagent	1. Weigh out 0.958g of SPADNS dye.	1a. i.e., 2(para sulfophenylazo) -1.8-dihydroxy -3,6- naphthalene disulfonate, also called 45 dihydroxy -3-(parasulfophenylazo) -27-napthalene disulfonic acid trisodium salt. 1b. Use an analytical balance and a plastic weighing boat.	
	2. Add about 200 ml of distilled water to a 1 liter volumetric flask.		
	3. Transfer the SPADNS dye to the 1 liter volumetric flask, using a powder funnel.	3a. Use a wash bottle to wash the solid into the flask. The weighing boat should be washed three times and the washings added to the flask.	
	4. Dissolve the SPADNS dye in distilled water.		
	5. Weigh out 0.133 gm zirconyl chloride octahydrate.	5a. Use a plastic weighing boat on the analytical balance.	
	6. Dissolve in about 200 ml distilled water.	6a. A 500 ml beaker may be used. 6b. Use a wash bottle to wash the solid into the flask. The weighing boat should be washed three times and the washings added to the flask.	
	7. Transfer the zirconyl chloride to the 1 liter flask with the SPADNS dye.	7a. Care should be taken to rinse the beaker with distilled water.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. SPADNS Reagent (Continued)	8. Add 350 ml concentrated hydrochloric acid (HCl).	8a. Caution: When using the concentrated acid, use eye protection. 8b. Solution may increase in temperature, cool to room temperature before proceeding.	
	9. Mix well.		
	10. Dilute to the 1 liter mark with distilled water.		
	11. Mix well.	11a, Solution stable for at least 2 years.	
	12. Label.		
3. Sodium Fluoride (NaF) stock solution - 100mg,F^-/1	1. Weigh out 0.2210 grams of sodium fluoride.	1a. Use an anlytical balance 1b. Use a plastic weighing boat. 1c. Solution can be purchased from Orion Research Inc: Cat No. 94-06-07 or Hach Chemical Co.; Cat No. 232-11.	
	2. Add about 500 ml of distilled water to a 1 liter volumetric flask.		
	3. Transfer the solid to the 1 liter volumetric flask using a powder funnel.		
	4. Use a wash bottle to wash solid into flask.	4a. The weighing boat should be washed three times and the washings added to the flask.	
	5. Dissolve the solid.		
	6. Dilute to the mark and mix thoroughly.	6a. Solution contains 0.1 mg F^-/1.0 ml (i.e. 100 ppm F^-). 6b. Keep in a plastic container. Solution is stable for at least 6 months.	
	7. Label		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Sodium Fluoride (NaF) Solution 10 mg F^-/1.	1. Add approximately 50 ml of distilled water to a 100 ml volumetric flask.		
	2. Pipet 10 ml of the stock fluoride solution (reagent 3) into the flask.	2a. Use a 10 ml volumetric pipet.	
	3. Dilute with distilled water to the mark.	3a. Solution contains 0.01 mg F/1.0 ml (i.e. 10 ppm).	
	4. Mix thoroughly.	4a. Keep in plastic container. 4b. Solution stable for at least 6 months.	
	5. Label		
5. Sodium Fluoride (NaF) Solution 1.0 mg F^-/liter	1. Add approximately 50 ml of distilled water to a 100 ml volumetric flask.		
	2. Pipet 10 ml of the fluoride solution (reagent 4) into the flask.	2a. Use a 10 ml volumetric pipet.	
	3. Dilute with distilled water to the mark.	3a. Solution contains 0.001 mg F^- per 1.0 ml (i.e. 1.0 ppm F^-).	
	4. Mix thoroughly.	4a. Solution should be prepared fresh daily.	
	5. Label		
6. Sodium Arsenite ($NaAsO_2$) Solution.	1. Weigh out 2.5 grams of sodium arsenite.	1a. A trip balance can be used.	
	2. Transfer the solid to a 500 ml Erlenmeyer flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
6. Sodium Arsenite ($NaAsO_2$) Solution (Continued)	3. Measure out 500 ml distilled water.	3a. Use a graduated cylinder.	
	4. Dissolve the sodium arsenite in about half of the distilled water.		
	5. Add the rest of the 500 ml portion.		
E. Calibration			
1. By use of a standard curve.	1. Turn on spectrophotometer and allow to warm up.	1a. Refer to manufacturer's manual for warm up time. However usually one-half hour is sufficient	
	2. Calibrate the spectrophotometer.	2a. This can be carried out in two ways: by use of a calibration graph or by calculation of the unknown. Section E.1 covers the calibration graph and Section E.2 covers the calculation.	VII E.1. (p. 21)
	3. Prepare a series of standard solutions using the 10mg F^-/liter sodium fluoride solution (Reagent D.4).	3a. The series of standards should be prepared in 50 ml volumetric flasks by pipetting with a graduated pipet the indicated amount of Reagent D.4 into a 50 ml volumetric flask and diluting to the volume mark,	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Calibration (Continued)		3b. The following table can be used to prepare a series of standards containing from 0 to 1.4 mg F^-/liter.	

ml of Reagent D.4	Conc. when diluted to 50 ml. mg F^-/liter
0.00	0.00
1.00	0.20
2.00	0.40
3.00	0.60
4.00	0.80
5.00	1.00
6.00	1.20
7.00	1.40

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	4. Transfer the entire 50 ml of the standards just prepared into 125 ml Erlenmeyer flasks.	4a. All eight standards can be prepared at the same time.	
	5. Add 10 ml SPADNS Reagent (Reagent D.2.).	5a. Use a 10 ml volumetric pipet 5b. Caution: This volume is critical; take care to add exactly 10 ml.	VII.E.1.5b (p. 22)
	6. Mix thoroughly.	6a. Unless mixed, the solution can layer giving false readings.	
	7. Determine the absorbance at 570 nm.	7a. The manufaturer's manual on operation of the spectrophotometer should be consulted for proper instrument operation.	V.E.1.7a. (p. 19)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Calibration (Continued)			
	8. Prepare a calibration curve.	8a. Use the attached graph paper. 8b. Refer to: Effluent Monitoring Procedure: Preparation of Calibration Graphs.	V.E.1.8 IX.E.1.8a (p. 19)
	9. Analyze the sample	9a. Operating Procedure F.	
	10. Obtain concentration using the graph prepared here.		
2. By Calculation	1. Turn on spectrophotometer and allow to warm up.		
	2. Adjust the wavelength to 570 nm.		
	3. Prepare two standards.	3a. Prepare as follows: 0 mg F^-/liter (pipet 50 ml of distilled water into a 125 ml Erlenmeyer flask): 1 mg F^-/liter (pipet 50 ml of reagent D.5 into a 125 ml Erlenmeyer flask).	
	4. Pipet 50 ml of each sample to be run into a series of 125 ml Erlenmeyer flasks.	4a. Label each flask with sample identification.	
	5. Pipet 10 ml of SPADNS Reagent to all flasks.	5a. This includes both standards (including 0 mg F^-/1) and samples.	
	6. Mix all thoroughly.		
	7. With nothing in the meter adjust the left side of the absorbance scale to read its maximum,	7a. This is the maximum (marked as infinity ∞).	
	8. Fill a sample cell with 0 mg F^-/liter standard.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. By Calculation (Continued)			
	9. Place the cell in the cell compartment.		
	10. Adjust the slit control so that a reading of 0.5 is obtained on the absorbance scale.	10a. This is to set the portion of the scale to be used.	VII.E.2.10a. (p. 22)
	11. Fill a sample cell with 1.0 mg F^-/liter standard.		
	12. Place in the cell compartment.		
	13. Read the absorbance value.	13a. This reading should read about 0.25 to 0.27. If this reading is not obtained the make up of the reagent and standard used should be checked.	
		13b. Use Operating Procedure F	
	14. Read all samples without changing any adjustment controls.		
	15. Calculate the concentration of the unknown.	15a. Use the equation: $X = \frac{A_o - A_x}{A_0 - A_1}$ X = concentration of the unknown in mg F^-/liter A_o= Absorbance reading of the 0 mgF^-/1. This is set in step 10 at 0.5	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. By Calculation (Continued)		15a. (continued) A. = Absorbance reading of the 1.0 mg F^-/liter (usually 0.25 to 0.27) A_X = Absorbance reading of the unknown.	
		15b. Example: If the sample absorbance value obtained from the spectrophotometer was 0.32 then $X = \frac{0.5-0.32}{0.5-0.25} = \frac{0.18}{0.25}$ $X = 0.72$ mg F^-/liter If more than one sample were run the only number that would change would be the 0.32. A calculation must be performed for each sample.	
F. Procedure	1. After calibration the samples are tested as follows.	1a. If the calibration graph was used (E.1.), two standards should be used each time to verify that the calibration curve is still valid. (A1.0 mg F^-/liter and a 0.2 mg F^-/liter). 1b. If the calculation procedure was used, the 0 and 1.0 ppm standards must be run with each batch of samples run.	
	2. Add 50 ml of sample to a 125 ml Erlenmeyer flask.	2a. Use a 50 ml volumetric pipet.	
	3. Add 10 ml SPADNS Reagent.	3a. Use a 10 ml volumetric pipet.	
	4. Mix thoroughly.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Procedure (Continued)			
	5. Read the absorbance value at 570 nm.		
	6. Dilute sample if concentration is not between 0.0 to 1.4 mg F^-/liter.	6a. If the calibration graph is used,the absorbance value for a concentration of 1.4 mg F^-/liter has been ploted do not use absorbance values corresponding to values greater than 1.4 mg F^-/liter. 6b. If the calculation procedure is used, obtain the absorbance value and calculate if the value calculated is above 1.4 mg F^-/liter dilute the sample.	

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX*	Records and Reports

*Training Guide materials are presented under the heading marked *. These standardized headings are used throughout this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
I.	The basis for the SPADNS procedure is the reaction between zirconium and the SPADNS dye (SPADNS is an abbreviation of sodium 2-(p-sulfophenylazo)-1,8-dihydroxy 3.6-napthlene disulfonate). The color of the reaction mixture (water sample plus reagent) varies from very deep red in the absence of fluoride to light red when the concentration of fluoride is high. The change in color caused by small changes in fluoride concentration is not discernable by eye, but can be readily detected by a photometric instrument. The better the instrument, the better the sensitivity to small changes of fluoride.	
B.2.	Distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary. However, manual distillation will be required to resolve any controversies. For drinking water samples analysis to comply with requirements as listed in the National Interim Primary Drinking Water Regulations (F. R. Part IV, 12/24/75-para. 141.23f,10-pg59573), distillation is necessary. Distillation of the calibration standards is not necessary should sample require distillation.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.1.7a	Should the analyst be using a Bausch and Lomb Spectronic "20" Spectrophtometer there is an EMP, "Use of a Spectrophotometer" availble. It would be of value to the analyst to consult this procedure.	
E.1.8	There is an EMP "Preparation of Calibration Graphs" that would be of value to the student.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

B.2.a

Other ions, particularly phosphate, iron and aluminum can cause significant errors. The table below gives some indication of this error. Should any of these interferences be present in the sample at concentrations that will cause error, the sample must be distilled. The numbers given show the concentration in mg/liter that will cause an error of plus or minus 0.1 mg/liter at 1.0 mg F^-/1.

Substance	Conc mg/liter	Type Error
Alkalinity ($CaCO_3$)	5000	-
Aluminum (Al^+)	0.1*	-
Chloride (Cl^-)	7000	+
Iron (Fe^{+3})	10	-
Hexametaphosphate ($[NaPO_3]_6$)	1.0	+
Phosphate (PO_4^{-3})	16	+
Sulfate ($(SO_4)^{-2}$)	200	+
Chlorine	Must be completely Removed with Arsenite	
Color & Turbidity	Must be Removed or Compensated for.	

Fluoride Determination in Water, E.P.A., Training Manual NTOTC, Cincinnati, OH 45268.

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*Above figure is for immediate reading. Allowed to stand two hours tolerance is 3.0 mg/liter, four hours tolerance is 30 mg/liter.

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

B.2.a. Continued

The temperature of the samples and standards must be the same before carrying out the test. Consequently if a difference in temperature exists, allow both to sit at room temperature for about one hour before proceeding with the test.

Analytical results obtained with the SPADNS reagent are limited to a range of 0 to 1.4 mg F$^-$/liter. Samples which approach or exceed the limit of the range must be diluted. The dilution must be made before addition of reagent, since subsequent dilution will affect the concentration of dye, zirconium and acid.

E.1.2a The first method to calibrate the spectrophotometer is to prepare a calibration graph. Calibration graphs are commonly used in absorbance measurements. In this type measurement energy is absorbed by some chemical constituent in the solution by means of a calibration graph.

Two things must be done in order to prepare a calibration graph. A series of standards must be prepared. A standard is a solution which contains a known amount of the same chemical constituent which is being determined in the sample. Secondly, the absorbance of these standards must be measured.

This is done by carrying the standards through the test procedure and measuring the amount of energy absorbed. This value is graphed against the known concentration and a line drawn through the points produced. This line is the calibration graph. When an unknown sample is run, its absorbance value is determined and using the calibration graph, its concentration can be determined.

The second method used in the SPADNS uses the fact that a straight line will be formed between 0 and 1.4 mg F/liter. Then the equation for a straight line is used to calculate the unknown concentration after values for two standards have been run.

This is carried out as follows. Two standards are prepared, usually 0 and 1 ppm. These are used to set the instrument, thus obtaining values for their absorption. These two knowns can be used to calculate the unknown by the equation. Both methods are shown in the EMP.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.1.5b	The addition of the highly colored SPADNS reagent must be done with utmost accuracy because the fluoride concentration is measured as a difference of absorbance in the standard and the sample. A small error in reagent addition is the most prominent source of error in this test after the interferences.	Methods for Chemical Analysis of Water and Wastes 1974, E.P.A. Environmental Monitoring and Support Laboratory Cincinnati, OH 45268.
E.2.10a	The absorbance scale is logarithmic. This means that the distance between numbers varies. The meter distance between numbers is large on the right side of the meter while the distance between numbers is small on the left side. For example the distance between 0 and 0.5 is over half the scale while from 1 to 2 is less than a quarter of the total distance. Consequently the right side of the scale can be read with a much higher degree of sensitivity and accuracy. The ratio of fluoride concentration and absorbance is inverse, that is the higher the fluoride concentration the lower the reading and the lower the fluoride concentration the higher the reading. Thus by setting the lowest concentration of fluoride possible, i.e., 0 mg F^-/liter, at 0.5 absorbance units this means that no reading can go higher. This restricts all readings to the most sensitive section of the scale.	

TYPICAL LABORATORY DATA SHEET

Sample Collection:

1. Name of Plant:____
2. Sampling Location:______________________________________
3. Type of Sample:____
4. Date and Time Collected:__________________________________
5. Sample Collector:______________________________________

Sample Analysis:

1. Name of Laboratory:____________________________________
2. Date and Time Collected·________________________________
3. Sample Designation:____________________________________
4. Method Used:____
5. Was Sample Distilled:___________________________________
6. Concentration of F^- Determined (in mg/liter):____________________

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF FLUORIDE IN POTABLE AND WASTEWATERS USING A SELECTIVE ION ELECTRODE

as applied in

WATER TREATMENT FACILITIES

WASTE WATER TREATMENT FACILITIES

and in the

MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.HAL.f.lab.WMP.2.11.77

EFFLUENT MONITORING PROCEDURE: Determination of Fluoride in Potable and Waste Waters Using a Selective Ion Electrode

This operational procedure was developed by:

NAME John D. Pfaff

ADDRESS USEPA, OWPO, National Training and Operational Technology Center
Cincinnati, Ohio 45268

POSITION Chemist - Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.A. Chemistry

3 years Research Chemist

14 years DHEW, ECA, EPA - Chemist

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EFFLUENT MONITORING PROCEDURE: Determination of Fluoride in Potable and Waste Waters Using a Selective Ion Electrode

1. Analysis Objectives:

The learner will determine the fluoride content of a water or wastewater sample using a selective ion (fluoride) electrode.

2. Brief Description of Analysis:

The pH of the sample is adjusted to between 5 and 5.5 by use of buffer solution. Then the electrodes are immersed in the solution and the fluoride content determined using the meter. If an expanded scale pH meter is used the concentration is read from a standard curve. If a specific ion meter is used the concentration can be read directly from the face of the meter. Other acceptable methods not covered in this outline are the SPADNS and automated complexone procedures.

3. Applicability of this procedure:

a. Range of Concentration:

from 0.1 to 1000 mg/l
Information is given so the same stepwise procedure can be used for fluoride concentrations up to 1000 mg/liter.

b. Pretreatment of Samples:

Distillation is not necessary for potable water samples. However, the guidelines for NPDES procedures specify distillation of waste water samples must be carried out. (See training guide note VII.B.1.1a.). The distillation procedure is not included but is covered in another EMP on Fluoride Distillation.

c. Treatment of Interferences in Samples:

Interferences are few when the buffer is used with the electrode. The polyvalent cations of si^{+4}, fe^{+3}, al^{+3}, interfere but can be tolerated up to 5.0 mg/l with use of the buffer. Extremes of pH can cause problems but adjustment of pH with the buffer negates this problem.

Additional information is given in the training guide note VII.E.4.

*Source of Procedure: Methods of Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, p.65.

EFFLUENT MONITORING PROCEDURE: Determination of Fluoride in Potable and Waste Waters Using a Selective Ion Electrode

Equipment and Supply Requirements

A. Capital Equipment:

1. Expanded scale pH meter or selective ion meter

   Examples:
   Beckman: Expandomatic Model 76007
   Coleman: Model 37A
   Corning: Model 12
   Fisher Accumet Model 320
   Leeds & Northrup Model 7405 - A 2.
   Orion Model 407A portable
   Hach pH/Fluoride meter No. 12330
   No. 12320 - portable

2. Sleeve-type reference electrode

   Examples:
   Beckman: No. 40463
   Coleman: No. 3-721
   Corning: No. 476012
   Fisher: No. 13-639-62
   Orion: No. 90-01

3. Fluoride Electrode

   Example:
   Beckman: 39600
   Coleman: 3-803
   Corning: 476042
   Orion: 94-09
   Orion: 96-09
   Hach: 13034-00

4. Trip balance, 500 gram capacity.

5. Magnetic stirer and teflon covered stir bar, about 2.5 cm long.

6. Water still or other source of distilled water.

B. Reusable Supplies:

1. One stop watch, clock or watch (with second hand).
2. One thermometer, glass 0 to 100°C.
3. One plastic squeeze bottle
4. One stirring rod, glass about 10 inches long.
5. One pair, safety glasses
6. One powder funnel, glass about 3 inch diameter
7. One laboratory apron
8. Five weighing boats, plastic (2-3 inches square).
9. One note book (for recording data)
10. One pen or pencil
11. One Flask volumetric 1000 ml volume.
12. Two Flask volumetric 100 ml volume
13. Ten Flasks 50 ml volume (for use with pH meter only)
14. One Cylinder graduated 500 ml volume
15. One Cylinder graduated 100 ml volume
16. Seven Pipet volumetric 10 ml
17. Two Pipet graduated 10 ml
18. Four Beakers, plastic 100 ml volume
19. One Pipet Bulb
20. One spatula

The following will be needed in addition to the above only if the buffer is prepared rather than purchased.

21. One Erlenmeyer flask, 500 ml volume
22. One Beaker, 1000 ml volume
23. One Flask volumetric, 1000 ml volume
24. One pH electrode

C. Consumable Supplies:

1. a. Sodium Fluoride -NaF Reagent grade powder - 4 oz.or
   b. Sodium Fluoride Stock Solution -
   Orion Research Inc. 380 Putnam Ave. Cambridge, Mass 02139, Cat. No. 94-06-07
   Hach Chemical Co. P.O. Box 907 Ames Iowa, 50010, Cat. No. 232-11

2. Adjustment buffer

   a. Total Ionic Strength Adjustment Buffer (TISAB)
   Orion Research Inc. 380 Putnam Ave. Cambridge, Mass. 02139 Cat. No. 94-09-09

   b. Fluoride Adjustment Buffer, Formula - 2589
   Hach Chemical Co. P.O. Box 907 Ames Iowa, 50010 Powder - Cat. No. 2589.01
   Pillows - Cat. No. 2589-99

C. Consumable Supplies (Continued):

3. The following are needed if the adjustment buffer is prepared instead of purchased.

   a. Acetic Acid, Glacial, CH_3COOH. Reagent grade - 1 pt
   b. CDTA* (1,2. cyclohexylene dinitrilotetraacetic acid) 25 g.
      Matheson, Coleman & Bell Cat. No. CX - 2390
   c. Sodium Chloride, NaCl, Reagent grade - 1 pound
   d. Sodium Hydroxide, NaOH, Reagent grade - 1 pound

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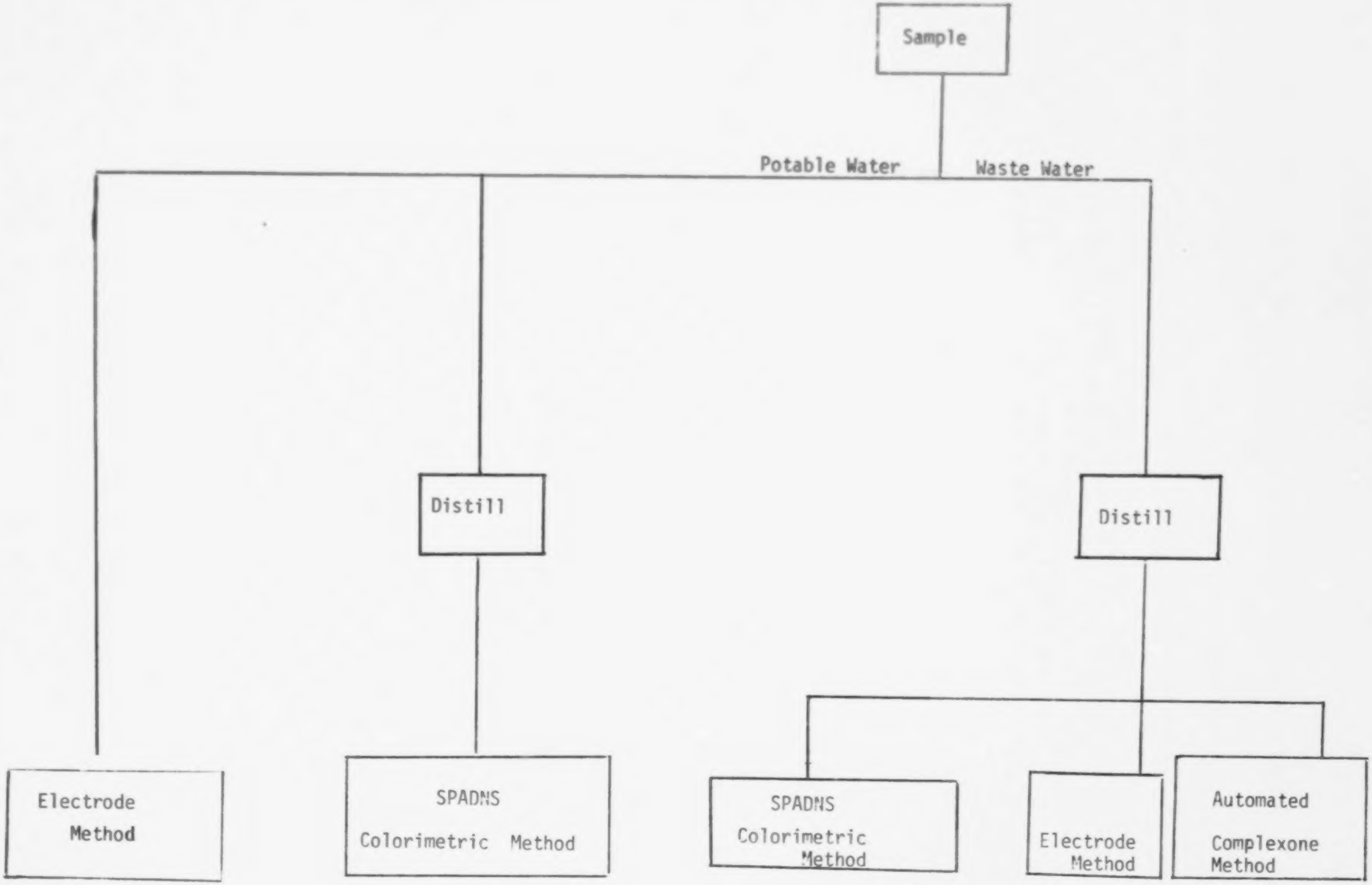
* Also listed as 1,2 cyclohexylene diaminetetraacetic acid Baker Cat. No. G083.

FLUORIDE ANALYSIS FLOW SHEET

E11.P-8


Sample
Potable Water
Waste Water
Distill
Distill
Electrode Method
SPADNS Colorimetric Method
SPADNS Colorimetric Method
Electrode Method
Automated Complexone Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Sample Collection	1. Collect a minimum of 300 ml in a plastic or hard glass container.	1a. Polyethylene bottles are preferred Glass bottles are satisfactory provided they have not previously contained high-fluoride solutions. 1b. No special requirements are necessary for preservation. 1c. Chlorine does not interfere so no precautions are necessary.	
B. Sample pretreatment	1. Distill sample	1a. Distill wastewater sample for total or total dissolved fluoride. 1b. Use the EMP covering the distillation procedure for Fluoride. 1c. Distillation is not required for drinking water samples.	
C. Equipment Preparation			
1. Glassware	1. Clean all glassware and plastic beakers in detergent.		
	2. Rinse with distilled water.		
2. Electrometer (pH meter or selective ion meter)	1. Check meter zero.	1a. Most instruments have a mechanical screw adjustment to center the pointer of the meter face.	
	2. Check power supply.	2a. Check correct connection to voltage source for line operated meters. Check batteries on all portable and some line operated models. See instrument manual for directions.	
	3. Check connection of electrodes to meter.	3a. There should be two connecting pins that attach to the meter. The longer one from the measuring electrode, is the fluoride electrode, and the smaller one is from the reference electrode.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		3b. Some connections are spring loaded and must be held in the socket until clamped with a screw. 3c. Combination electrodes are two electrodes placed one inside the other. This electrode will still have two connecting pins.	
3. Reference Electrode	1. Check Internal filling solution.	1a. Before using the reference electrode make sure the internal filling solution has been added. This solution is provided with the electrode and must be used. 1b. Fill about half the electrode with the solution.	
D. Reagent Preparation			
1. Distilled Water	1. Prepare about six (6) liters of distilled water. This water must be free from fluoride.	1a. Use a still or pass tap water through an ion-exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin.	
2. Sodium Hydroxide NaOH, 5N (for use in preparation of buffer)	1. Weigh out 40 grams of sodium hydroxide (NaOH)	1a. Use a trip balance. 1b. Put sodium hydroxide in a plastic weighing boat for weighing.	
	2. Dissolve the sodium hydroxide in 200 ml of water in a 500 ml Erlenmeyer flask.	2a. Use a graduated cylinder to measure the 200 ml of water. 2b. Caution: heat given off.	
3. Buffer Solution (preparation optional - can be purchased)	1. Add approximately 500 ml of distilled water to a 1 liter beaker.	1a. This solution can be purchased already prepared. Sources are listed in the front of the EMP.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Reagent Preparation			
3. Buffer Solution (Continued)	2. Add 57 ml of glacial acetic acid.	2a. Use a graduated cylinder. 2b. Caution: Use in a well ventilated area. 2c. Use safety glasses.	
	3. Weigh out 58 grams of sodium chloride (NaCl).	3a. Use a trip balance. 3b. *Put sodium chloride in a plastic weighing boat* for weighing. 3c. For work with brines additional NaCl should be added to raise the chloride level to twice the highest expected level of chloride in the sample.	
	4. Add sodium chloride (NaCl) to the 1 liter beaker.	4a. That is the same beaker to which the glacial acetic acid has been added.	
	5. Weigh out 4 grams of CDTA.	5a. The E.P.A. Methods Manual 1974, calls for 2 grams; however, STD Methods 14th and the Orion manual both call for 4 grams. 5b. Use trip balance. 5c. Put CDTA in a plastic weighing boat for weighing. 5d. CDTA - 1,2 cyclohexylene diamine tetraacetic acid.	
	6. Add CDTA to the 1 liter beaker.		
	7. Stir to dissolve.		
	8. Place beaker in a cool water bath.	8a. Cool to room temperature.	
	9. Insert calibrated pH electrode into solution.	9a. The same meter that will be used for the fluoride measurement can be used for this. 9b. Consult EMP on pH measurement.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Reagent Preparation 3. Buffer Solution (Continued)	10. Add 5N Sodium hydroxide (Reagent D2) (NaOH) to the solution until meter reads 5.0 to 5.5 pH.	10a. Reagent 2. 10b. About 150 ml will be needed. 10c. Use a 100 ml graduated cylinder to add the sodium hydroxide until the pH nears 5.0. Then add from a pipet dropwise until the range of 5.0 to 5.5 is reached.	
	11. Allow solution to reach room temperature.		
	12. Transfer solution to a 1 liter volumetric flask.	12a. Use wash bottle and rinse beaker with small amounts of distilled water, transferring this wash water to the volumetric flask.	
	13. Dilute to volume and mix thoroughly.		
4. Sodium Fluoride (NaF) stock solution 100 mg F^-/liter	1. Weigh out 0.2210 grams of sodium fluoride.	1a. Use analytical balance. 1b. Use plastic weighing boat. 1c. Solution can be purchased from Orion Research Inc., Cat NO. 94-06-07 or Hach Chemical Co., Cat. No. 232-11	
	2. Add about 500 ml of distilled water to a 1 liter volumetric flask.	2a. Approximately ½ full.	
	3. Transfer the solid to the 1 liter volumetric flask using a powder funnel.		
	4. Use wash bottle to wash the solid into flask.	4a. The weiging boat should be washed three times and the washings added to the flask.	
	5. Dissolve the solid.		
	6. Dilute to volume and mix thoroughly.	6a. Solution contains 0.1 mg F^- per 1.0 ml (i.e. 100 ppm F^-)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Reagent Preparation		6b. Keep in plastic. Stable for 6 months.	
4. Sodium Fluoride (NaF) stock solution 100 mg F^-/liter (Continued)	7. Label container.		
5. Sodium Fluoride (NaF) Solution 10 mg F^-/liter	1. Add approximately 50 ml distilled water to 100 ml volumetric flask.		
	2. Pipet 10 ml of the (NaF) stock solution (reagent C.4.) into the flask.	2a. Use a 10 ml volumetric pipet.	
	3. Dilute with distilled water to the mark.	3a. Solution contains 0.01 mg F^- per 1.0 ml i.e.,10 ppm. Label solution.	
	4. Mix thoroughly.		
6. Sodium Fluoride (NaF) solution 1.0 mg F^-/liter	1. Add approximately 50 ml of distilled water to a 100 ml volumetric flask.		
	2. Pipet 10 ml of reagent 5 (10 mg F^-/1) liter into the flask.	2a. Use a 10 ml volumetric pipet.	
	3. Dilute with distilled water to the mark.	3a. Solution contains 0.001 mg F^- per 1.0 ml i.e., 1.0 ppm.	
	4. Mix Thoroughly.	4a. Label	
E. Meter and Electrode Check	1. Turn on Meter		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Meter and Electrode Check (Continued)	2. Allow to warm up.	2a. If battery powered, no warm up necessary.	
	3. Pipet 10 ml of Reagent 6 into a 100 ml plastic beaker.	3a. Use a 10 ml volumetric pipet.	
	4. Add 10 ml of buffer i.e., Reagent 3.	4a. Use a 10 ml volumetric pipet. 4b. Any volume of sample or standard and buffer can be used, provided equal volumes are used. For example 10 ml of sample plus 10 ml of buffer. 4c. Use of powdered buffer (Hach Co.) eliminates the dilution of sample or standard and therefore eliminates possible error. 4d. Volume should be sufficient to cover the electrode or electrodes to a depth of about 1 inch.	VII.E.4 VII.E.4b (pp. 27 & 28)
	5. Place a stirring bar into the beaker.		
	6. Place beaker on stir plate.		
	7. Lower electrode or electrodes into solution.	7a. Care should be taken that the stirring bar does not hit the electrode. 7b. Before using the reference electrode make sure the internal filling solution has been added. This solution is provided with the electrode and must be used. Fill about half the electrode with the solution.	V.E.7. (p. 26)
	8. Turn on stir plate.	8a. For best results stirring should be at a rate that will not cause a vortex. 8b. Insulate with cardboard or styrofoam between sample and stir plate to reduce possibility of sample temperature change. Not so thick as to stop the stirrer.	
	9. Turn meter on to a millivolt reading position.	9a. The meter should be used in the expanded scale mode.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Meter and Electrode Check (Continued)	10. Record reading in millivolts.	10a. The electrodes must remain in the solution for at least three minutes or until the reading has stabilized. At concentrations under 0.5 mg F^-/liter it may require as long as five minutes to reach a stable meter reading. Higher concentrations stabilize more quickly.	
	11. Turn meter to standby position or off position.	11a. Depends on type meter being used.	
	12. Raise electrodes from solution.		
	13. Rinse electrodes with distilled water.		
	14. Blot dry with soft tissue.		
	15. Pipet 10 ml of Reagent 5 into a clean 100 ml plastic beaker.	15a. Use a 10 ml volumetric pipet.	
	16. Add 10 ml buffer.	16a. Use a 10 ml volumetric pipet. 16b. Samples and standards should be at the same temperature. A 1°C difference in temperature will give rise to about a 2 % error.	
	17. Place a stirring bar into the beaker.		
	18. Place beaker on stir plate.		
	19. Lower electrodes into solution.	19a. Care should be taken that the stir bar does not hit the electrode.	
	20. Turn meter on at a millivolt reading position.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Meter and Electrode Check (Continued)	21. After 3 minutes record the reading in millivolts.	21a. Or until the meter stabilizes.	
	22. Determine the difference between the first and second millivolt reading.	22a. Correct electrode operation is indicated by a difference of about 58 millivolts, assuming the solution temperature is between 20°C and 25°C. 22b. If the change is not within ± 2 millivolts, consult the electrode manual.	III.E.22 (p. 25)
F. Calibration			
1. Using a pH meter	1. Turn on meter and allow to warm up.		
	2. Prepare a series of standards using Reagent 5.	2a. The following table can be used to prepare a series containing from 0 to 2.0 mg F^-/liter by diluting appropriate volumes to 50 ml.	

ml of Reagent 5	Conc. when diluted to 50 ml in mg F^-/liter
0.00	0.00
1.00	0.20
2.00	0.40
3.00	0.60
4.00	0.80
5.00	1.00
6.00	1.20
7.00	1.40
8.00	1.60
10.00	2.00

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		2b. Use a 10 ml graduated pipet.	
	3. Pipet 10 ml of a standard into a 100 ml plastic beaker.	3a. Any volume can be used provided equal amounts of sample or standard and buffer are used.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration			
1. Using a pH meter (Continued)		3b. Use a 10 ml volumetric pipet.	
	4. Add 10 ml of buffer.	4a. Use a 10 ml volumetric pipet.	
	5. Add a stir bar to the beaker.		
	6. Lower the electrodes into the beaker.		
	7. Turn on stir plate.	7a. Do not allow stir bar to hit electrode. 7b. Stir at a rate that will not cause a vortex.	
	8. Turn on meter.	8a. Use expanded mode. 8b. Use millivolt mode.	
	9. Allow meter to stabilize.	9a. About 3 minutes.	
	10. Record millivolt reading.		
	11. Turn meter to off or standby position.	11a. Always go to either position, depending on meter, before raising the electrodes from the solution.	
	12. Raise electrodes.		
	13. Rinse the electrodes.	13a. With distilled water.	
	14. Blot dry.	14a. With soft tissue.	
	15. Repeat steps 3-13 for each standard.	15a. Repeat for each standard until all are run, recording the millivolt reading.	
	16. Prepare a standard curve.	16a. Plot on two cycle semilog paper.	VII F.1.15a
2. Using a selective Ion Meter	1. Pipet 10 ml of Reagent 6 into a 100 ml plastic beaker.	1a. Use a 10 ml volumetric pipet.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration 2. Using a selective Ion Meter (Continued)		1b. Any volume can be used provided equal amounts of sample or standard and buffer are used.	
	2. Pipet 10 ml of buffer into the beaker.	2a. Use a 10 ml volumetric pipet.	
	3. Place a stirring bar into the beaker.		
	4. Place beaker on stir plate.		
	5. Lower electrodes into beaker.	5a. Do not allow stir bar to hit electrode.	
	6. Turn on stir plate.	6a. Stir at a rate that will not cause vortex.	
	7. Turn meter to the monovalent anion position.	7a. This may be marked as X^- or F^-. 7b. Allow three minutes before reading.	VII.F.2.8a. (p. 29)
	8. Using the calibration control adjust the meter to read at center scale.	8a. Use the logrithmic scale on the meter. 8b. This adjustment makes the instrument read a concentration of 1.0 mg F^-/liter at center scale.	
	9. Turn meter to off.		
	10. Raise the electrodes.		
	11. Rinse electrodes with distilled water.		
	12. Blot dry with soft tissue.		
	13. Pipet 10 ml of Reagent 5 into a clean plastic 100 ml beaker.	13a. Use a 10 ml volumetric pipet. 13b. This solution will be a 10 mg F^-/liter standard.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration			
2. Using a selective Ion Meter (Continued)	14. Add 10 ml of buffer.	14a. Use a 10 ml volumetric pipet.	
	15. Place stir bar into beaker.		
	16. Place beaker onto stir plate.		
	17. Lower electrodes into solution.	17a. Do not allow stir bar to hit electrodes.	
	18. Turn meter to monovalent anion position.		
	19. Allow to stir for three minutes.	19a. Or until meter stabilizes.	
	20. Use Temperature compensator to adjust meter needle to the last number on far right of log scale.	20a. The temperature of all samples and standards should be the same. If sample is not the same temperature, allow to stand at room temperature for about one hour. 20b. This adjustment makes the meter read a concentration of 10.0 mg F^-/liter at far right scale. 20c. The entire scale now can be used to read directly, concentrations from 0.1 mg F^-/liter to 10.0 mg F^-/liter. 20d. This calibration should be checked about every 1 to 2 hours by repeating procedure F2. If the temperature changes, recalibrate more often. 20e. The log scale may be located at various positions on the meter face. Consult the manufacturers manual to point out which to use if in doubt.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calibration			
2. Using a selective Ion Meter (Continued)	21. Turn meter off.		
	22. Raise electrodes.		
	23. Rinse electrodes with distilled water.		
	24. Blot dry with soft tissue.		
G. Procedure	1. After the calibration of the meter has been completed, test all samples by doing the following.		
	2. Turn on meter for warm up.	2a. If necessary.	
	3. Pipet 10 ml of sample into a clean plastic 100 ml beaker.	3a. Any volume can be used provided equal amounts of sample or standard and buffer are used. 3b. Use a 10 ml volumetric pipet.	
	4. Add 10 ml of buffer.	4a. Or same volume as used for sample.	
	5. Place stir bar into beaker.		
	6. Place beaker on stir plate.		
	7. Lower electrode.		
	8. Turn on stir plate.	8a. Do not allow stir bar to hit electrode.	
	9. Adjust stir plate to a rate that will not form vortex.		
	10. Turn on meter.	10a. To monovalent anion position.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Procedure (Continued)	11. Stir for three minutes, or until meter stabilizes.		
	12. Read value.	12a. For pH meters read at millivolt scale and use millivolt vs concentration curve to determine F^- concentration in sample. 12b. For selective ion meter read concentration directly from logarithmic scale.	
	13. Turn meter off.		
	14. Raise electrodes.		
	15. Rinse and dry electrodes.		
H. Storage			
1. Reference Electrode	1. Store in the 1.0 mg F^-/liter standardizing solution or distilled water if the electrode is to be used in a short time or next day.	1a. If using separate electrodes, i.e. reference and fluoride, this section applies to the reference only. If a combination electrode is being used the precautions for the reference electrode section should be used.	
	2. Clean thoroughly and store dry if storing for long period of time.	2a. Before reuse add internal filling solution.	
2. Fluoride Electrode	1. Store in air or in a standardizing solution.	1a. No specific storage precautions necessary.	
I. Calculations			
1. pH meter	1. Use the constructed standard curve.	1a. When the reading of millivolts is obtained use this value to convert to concentration in mg F^- liter.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Calculations			
1. pH meter (Continued)		1b. Using the graph paper attached. Look at the bottom of the graph and find the millivolt reading for the sample. Move upward until the curve is contacted then move horizontally left until the edge of the paper is reached. Read this intersection in mg F^-/ liter. 1c. If additional instruction on the use of a calibration graph is needed, consult the EMP, Preparation of Calibration Graphs.	
2. Specific Ion Meters	1. Read concentration scale (logarithmic directly to obtain sample mg F^-/liter.	1a. All data reported in mg F^-/liter.	

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII*	Field & Laboratory Analysis
VIII	Safety
IX*	Records & Reports

Training guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

INTRODUCTION Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	The basis for this method is in the fluoride electrode itself. Most electrodes contain a fluoride solution; at the tip of the electrode is a crystal doped with fluoride ions. The crystal acts as an ion-exchange membrane, so that when the fluoride concentration outside of the electrode is higher than that inside, ions move toward the inside setting up a voltage potential proportional to the difference in fluoride concentration. Conversely when the fluoride concentration on the outside is lower than that on the inside, a proportional potential of opposite sign is set up. In most fluoride electrodes, the internal solution is about 10^{-3} molar in fluoride, so concentrations below 19 mg F^-/liter result in positive voltage readings. Some electrodes contain no internal solution, but the principle of operation is similar. However, in the solid electrodes the potential developed by a particular fluoride solution is independent of a filling solution but rather depends entirely on the characteristics of the particular crystal used in the electrode manufacture.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.22	Theory of operation of a fluoride electrode predicts a behavior that is expressed by the Nernst equation. Consequently the difference in millivolt readings between two concentrations of fluoride that differ by a factor of ten should be between 55 and 60 millivolts. If one knows concentration of fluoride, for example 1 mg/liter, is expressed on a meter in millivolts and then a second concentration differing by a factor of ten, for example 10 mg/liter, is expressed on the same meter in millivolts, the difference between these millivolt values will be about 58 millivolts. This fact can be used to check on whether the system is operating properly. How this is carried out will depend on the type of meter and electrode being used.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.7	The fluoride electrode must be used in conjunction with a standard single junction sleeve-type reference electrode. If smaller volumes of sample are to be run for convenience sake, a combination electrode is available. This appears to be a single electrode. However, in actuality, it is two electrodes, one inside the other. This allows the electrode to be inserted into smaller diameter containers and also makes for easier use.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.1.1a.	For potable waters to comply with requirements as listed in the National Interim Primary Drinking Water Regulations (F.R. Part IV - 12/24/75-§10 - pg 59573), distillation is not necessary. However, for surface and saline waters or domestic and industrial wastes to comply with NPDES requirements distillation if necessary unless sufficient data exists to prove that distillation is not necessary. Manual distillation will be required to resolve any controversy.	
E. 4.	Interferences connected with the electrode can be listed as being: a) pH b) temperature c) other ions d) total ionic strength In acid solutions below pH 5, hydrogen can complex fluoride by forming the undissociated acid hydrofluoric HF. This will not allow the electrode to sense the fluoride concentration, thus tied up. Hydroxide ion can also interfere when concentration of this ion are about 10^{-7} Molar. Addition of the buffer solution will adjust most samples into the range of 5.0 to 5.5 pH where no interference of pH is found. For samples high in pH, i.e., pH 11, the volume of buffer added may not be sufficient. These samples should have their pH adjusted by the addition of 1N HCl to adjust the pH to about 8 before addition of the buffer. So long as the temperature of the standards and samples are the same, temperature will not affect the readings. However a variance between standard and sample of 1°C can cause a 2% error for a concentration of 19 mg F/l. Other ions, particularly, iron and aluminium can cause significant errors. The table below gives some indication of this error. The numbers given show the concentration that will affect the electrode after addition of buffer.	

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

E.4 (Continued)

INTERFERENCES FOR THE ELECTRODE

Fluoride Determination in Water, E.P.A. Training Manual, National Training and Operational Technology Center, Cincinnati, Ohio 45268

Concentration of substance, in mg/liter required to cause an error of plus or minus 0.1 mg/liter at 1.0 mg F/liter.

SUBSTANCE	
Alkalinity	7,000 (+)
Aluminum	4 (-)
Chloride	20,000 (-)
Iron	200 (-)
Hexametaphosphate	>50,000
Phosphate	>50,000
Sulfate	50,000 (-)
Chlorine	>5,000

From this, it can be seen that with the use of the buffer, very few ions normally used in the treatment of water will interfere.

Variations in ionic strength can interfere but again the make-up of the buffer will adjust this to a point where interferences are eliminated.

E.4.b. All samples are actually diluted in half by the addition of an equal volume of buffer solution. Since this same dilution ratio of equal volumes is carried out for all samples and standards the original concentration is used. Thus if a 1.0 mg/liter solution is diluted with buffer and is actually 0.5 mg/liter it is read as 1.0 mg/liter.

F.1.15a. Plot the concentration of fluoride in mg/l on the log axis and the millivolt reading on the linear axis. Figure one has a piece of 2 cycle semi log paper with the concentration values in the EMP marked.

The graph will have a negative slope; i.e., there will be a lowering of the millivolt reading as the concentration increases. The line will be straight from 0.1 to over 100 ppm.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.2.8a.	A selective ion meter usually has several scales on the meter face. One is divided into 14 equal divisions is the pH scale; another is divided into unequal divisions and usually repeats itself at least twice. How it is numbered varies with each manufacturer. This scale is the logarithmic scale and should be used to read the concentration of selective ions in mg/l. Since the units, i.e. how it is numbered, vary the concentration range of the scale can be arbitrarily set. This scale is usually repeated; for example, if the first number on the left is marked as .1 and at the middle of the scale is 1 and the last number on the right is 10 this gives two complete repetitions. Concentration wise this could mean that the concentration at center can be adjusted to read 1.0 mg/l and at far right as 10.0 mg/l. This would then allow concentrations of from 0.1 to 1.0 to 10.0 to be read directly from the meter face.	

TYPICAL LABORATORY DATA SHEET

Sample Collections:

1. Name of Plant: ______________________________
2. Sampling Location ______________________________
3. Type of Sample ______________________________
4. Date and Time Collected ______________________________
5. Sample Collector ______________________________

Sample Analysis:

1. Name of laboratory ______________________________
2. Date and Time Collected ______________________________
3. Sample designation ______________________________
4. Methods Used ______________________________
5. Was Sample Distilled ______________________________
6. Concentration of F^- determined (in mg/liter) ______________________________

Standard Conc. mg F^-/liter	Millivolt reading
0.0	
0.2	
0.4	
0.6	
0.8	
1.0	
1.2	
1.4	
1.6	
2.0	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

PRELIMINARY DISTILLATION PROCEDURE FOR FLUORIDE ANALYSIS OF POTABLE AND WASTEWATERS

as applied in

WATER TREATMENT FACILITIES
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.HAL.f.lab.WMP.1.11.77

WATER MONITORING PROCEDURE: Preliminary Distillation Procedure for Fluoride Analysis of Potable and Wastewaters

1. Analysis Objectives:

   The learner will remove interferences to the fluoride analyses by a preliminary distillation of the sample.

2. Brief Description of Analysis:

   The sample is added to a previously adjusted acid water mixture. The fluoride is liberated in the strongly acid mixture as the fluoride ion F^-. This reacts with the excess hydrogen ion H^+ to form hydrofluoric acid HF. This material leaches silica from glass and forms hydrofluorsilic acid $H_2S_1F_6$) which distills over as an aqueous solution. All impurities remain behind.

3. Applicability of this procedure:

   a. Range: When high-fluoride samples are distilled (greater than 10 mg F^-/l), all of the fluoride may not be distilled over. Repeat the distillation with 300 ml distilled water.

   b. Pretreatment: None required

   c. Treatment of Interferences: None required

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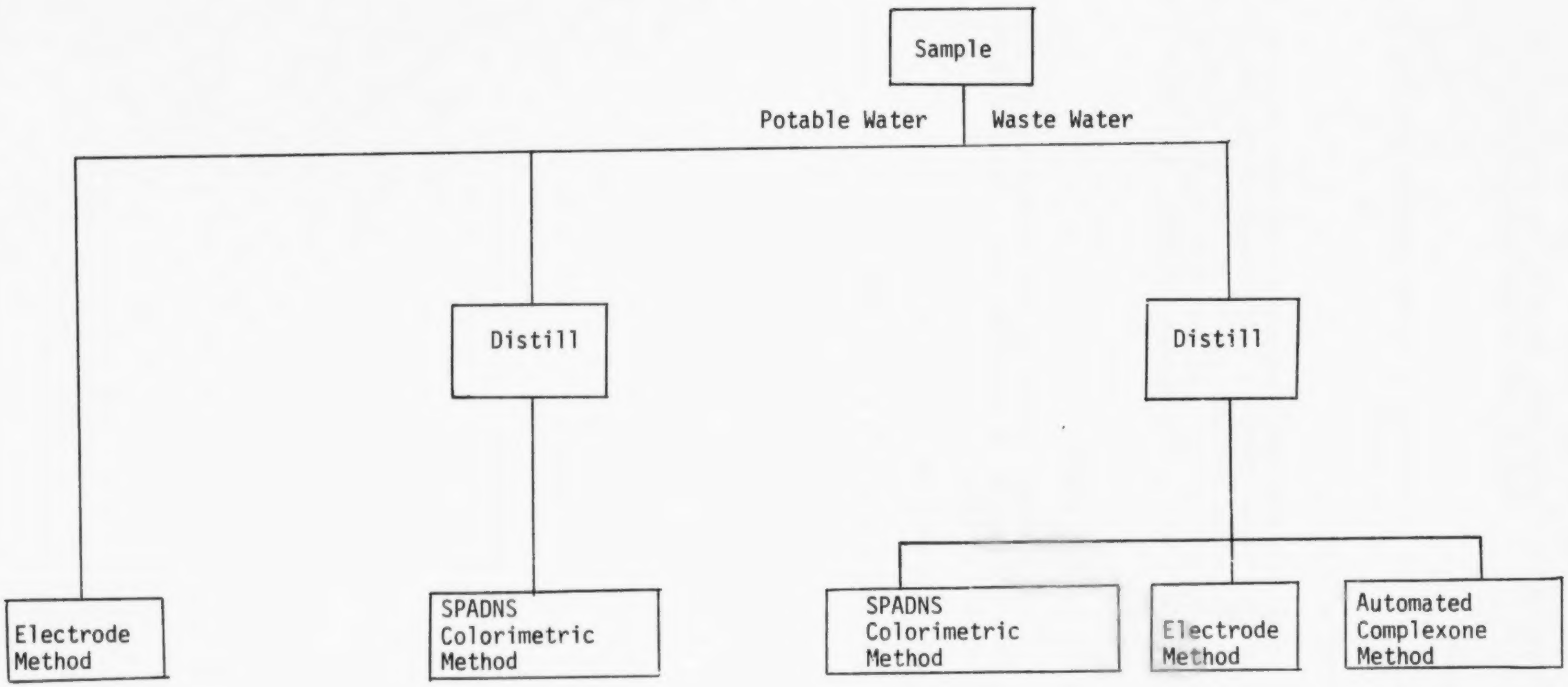
FLUORIDE ANALYSIS FLOW SHEET


Sample
Potable Water
Waste Water
Distill
Distill
Electrode Method
SPADNS Colorimetric Method
SPADNS Colorimetric Method
Electrode Method
Automated Complexone Method

WATER MONITORING PROCEDURE: Preliminary Distillation Procedure for Fluoride
Analysis of Potable and Wastewaters

Equipment and Supply Requirements

A. Capital Equipment:

Water still or other source of distilled water

B. Reusable Supplies:

1. One adaptor - offset with outer ground glass joint 24/40 at top and inner ground glass joint 24/40 at the bottom, with opening with rubber glove connector for thermometer.
2. One burner, natural - gas type (or for other type gas used in lab)
3. One boiling flask - 1 liter, Pyrex or Kimax, round bottom, with 24/40 outer ground glass joint
4. Two clamps - to hold boiling flask and condenser to ring stand. One should be covered with asbestos or fiberglas to withstand heat.
5. One condenser - 40 cm long, double jacket, outer ground glass 24/40 joint at top
6. One connecting tube - with two inner ground glass 24/40 joints
7. One cylinder - volumetric, 500 ml
8. One flask - Erlenmeyer, 300 ml
9. Twelve glass beads - (not hard glass, i.e., Pyrex or Kimax)
10. One tube grease - silicone stopcock
11. One ring stand and rod
12. One thermometer - 0 to 200°C, 24" long
13. Tubing, Tygon - enough to reach water supply and drain

C. Consumable:

1. Distilled water - about 6 liters
2. Silver sulfate - crystals (for removal of chloride concentration greater than 2000 mg/l)
3. Sulfuric acid
4. Detergent

WATER MONITORING PROCEDURE: Preliminary Distillation Procedure for Fluoride Analysis of Potable and Wastewaters

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
PRELIMINARY DISTILLATION	FOR FLUORIDE ANALYSIS:		
A. Sample Collection	1. Collect a minimum of 300 ml in a plastic or hard glass container.	1a. Polyethylene bottles are preferred to glass bottles and are satisfactory provided they have not previously contained high-fluoride concentration solutions. 1b. No special requirements are necessary for preservation.	
B. Equipment Preparation	1. Clean all glassware in detergent.		
	2. Rinse with distilled water.	2a. Caution: Tap waters can contain fluoride.	
C. Reagent Preparation 1. Distilled Water	1. Prepare about six (6) liters of distilled water. This must be free from fluoride.	1a. Use a still or pass tap water through an ion-exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin.	
2. Sulfuric Acid	1. Concentrated; no preparation required.		
D. Still Preparation 1. Connect the still	1. As shown in Fig. I.	1a. Leave the joint between the adaptor and connecting tube open.	
	2. The ground glass joints should be greased with silicone stopcock grease.	2a. Very small amounts are used. One way to do this is to coat the joint then lightly wipe the grease off with a soft tissue.	
	3. The thermometer must be capable of reading about 200°C.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Still Preparation (Continued) 2. Add the Reagents	1. Place 400 ml of distilled water in the boiling flask.	1a. Use a 500 ml graduated cylinder.	
	2. Carefully add 200 ml concentrated sulfuric acid.	2a. Caution: Heat is generated, also use protective eye covering.	
	3. Add about 10 to 12 glass beads.	3a. These serve a two fold purpose: first to act as boiling stones to prevent superheating of the mixed liquids and secondly to serve as a source of silica for a chemical reaction in the flask. $F^- + H \rightarrow HF + Si \rightarrow H_2SiF_6$ If the glass beads are not present, the silica will be taken from the boiling flask and will considerably shorten its useful life.	
	4. Shake the flask by swirling to mix the two liquids.	4a. Hold the ring stand top and rotate in circular fashion. The mixing must be complete or the liquid can superheat. If the flask is observed closely while mixing, gradient lines (wavy lines) can be seen. Mix until no more are seen.	VIII.2.D.4 (p. 15)
3. Close the Still	1. Connect the joint between the adaptor and the connector tube.	1a. Consult Figure I.	
	2. Check all joints to assure tightness.	2a. Fluoride will be lost through any joints that are not tight.	
4. Heat the Still	1. Turn on water to the condenser.	1a. The water should enter at the bottom of the condenser. 1b. The flow of water should be maintained at such a rate that condensation does occur in the upper third of the condenser.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Still Preparation (Continued)	2. Turn on and ignite the burner.	2a. A heating mantle may be used; however, it is inconvenient to use. The temperature must be stopped at 180°C and it is hard to do with a mantle. If a mantle is used, it can be turned off at a somewhat lower temperature, allowing the residual heat to carry the temperature to 180°C. How early should be determined by practice since all mantles very.	
	3. Place a 300 ml Erlenmeyer flask under the condenser.		
	4. Begin to heat slowly.	4a. If bumping occurs, mixing has not been completed.	
	5. When boiling begins, heating may be increased.	5a. Adjust the flame to prevent it from contacting the distilling flask above the liquid level. Superheating of the vapor results in high sulfate carryover which causes a sulfate interference.	
	6. Continue heating until the temperature reaches 180°C.	6a. Distillation must be stopped when the temperature reaches 180°C. Higher temperatures result in excessive sulfate carryover. 6b. About 45 minutes.	
	7. Remove heat.	7a. Turn off burner.	
	8. Discard distillate.	8a. Discard this distillate, since it contains traces of fluoride from the acid and glassware. This preliminary procedure also serves to adjust the acid-water ratio for subsequent distillations. 8b. This preliminary distillation need be repeated only if new acid is used or obvious contamination occurs or if the operator has doubts as to contamination. 8c. The resulting acid-water ratio can be used over and over for samples until a brown color predominates and 180°C is hard to obtain. 8d. The amount of distillate should be 300 ml.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Procedure 1. Sample Addition	1. Allow the still to cool until the temperature drops to 120°C or lower.	1a. Caution: The glass is hot; touch with care.	
	2. Disconnect the joint between the adapter and connecting tube.	2a. The connecting tube may be lifted clear of the adapter and condenser.	
	3. Remove high levels of chloride in the sample.	3a. Add silver sulfate to the sample at the rate of 5 mg per milligram of chloride when high-chloride samples are distilled. 3b. Most potable waters will not have high-chlorides. Sea water and brackish waters may have. As a generalized guide, about 2000 mg/l chlroide should be a starting point for adding the silver sulfate.	
	4. Add 300 ml sample.	4a. Use a 500 ml graduated cylinder. 4b. Pour into adapter.	
	5. Mix thoroughly.	5a. Caution: Improper mixing may cause super-heating and bumping. 5b. Use support ring and swirl in circular manner.	
	6. Close joint between adapter and connecting tube.		
2. Distill sample	1. Place a 300 ml Erlenmeyer flask under condenser.		
	2. Turn on water to the condenser.	2a. If it has been turned off.	
	3. Ignite burner.		

WATER MONITORING PROCEDURE: Preliminary Distillation Procedure for Fluoride Analysis of Potable and Wastewaters

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Procedure (Continued)	4. Begin to heat slowly.	4a. Until boiling begins.	
	5. Increase temperature.	5a. Caution: Do not allow flame to go above liquid level.	
	6. Heat to 180°C.		
	7. Remove heat.	7a. When temperature reaches 180°C.	
3. Remove Distillate	1. Retain distillate for analysis of the fluoride.	1a. When high-fluoride samples (> 10 mg F^-/liter) are distilled, repeat the distillation using 300 ml of distilled water. If substantial amounts of fluoride appear in the second distillate, add the amount to that obtained initially and flush (300 ml of water) the still again. Quantities of less than 0.1 mg/l F^- may be disregarded.	
F. Fluoride Determination	1. Chose an approved method.	1a. For wastewater the SPADNS-electrode or automated complexone methods are approved after distillation. 1b. For potable water the SPADNS or electrode methods are approved. Distillation is not required for the electrode method. 1c. Consult the appropriate EMP on the procedures.	
	2. Determine the fluoride concentration.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Storage			
1. The Still	1. Store the glass still in such a manner as to prevent physical damage.		
2. The Acid	1. Store in the still.	1a. This acid may be reused until the buildups of impurities discolors or prevents the temperature from being obtained. 1b. The time is dependent upon content of impurities in the water sample. 1c. A good practice would be to occasionally run a known standard to assure complete distillation of fluoride.	

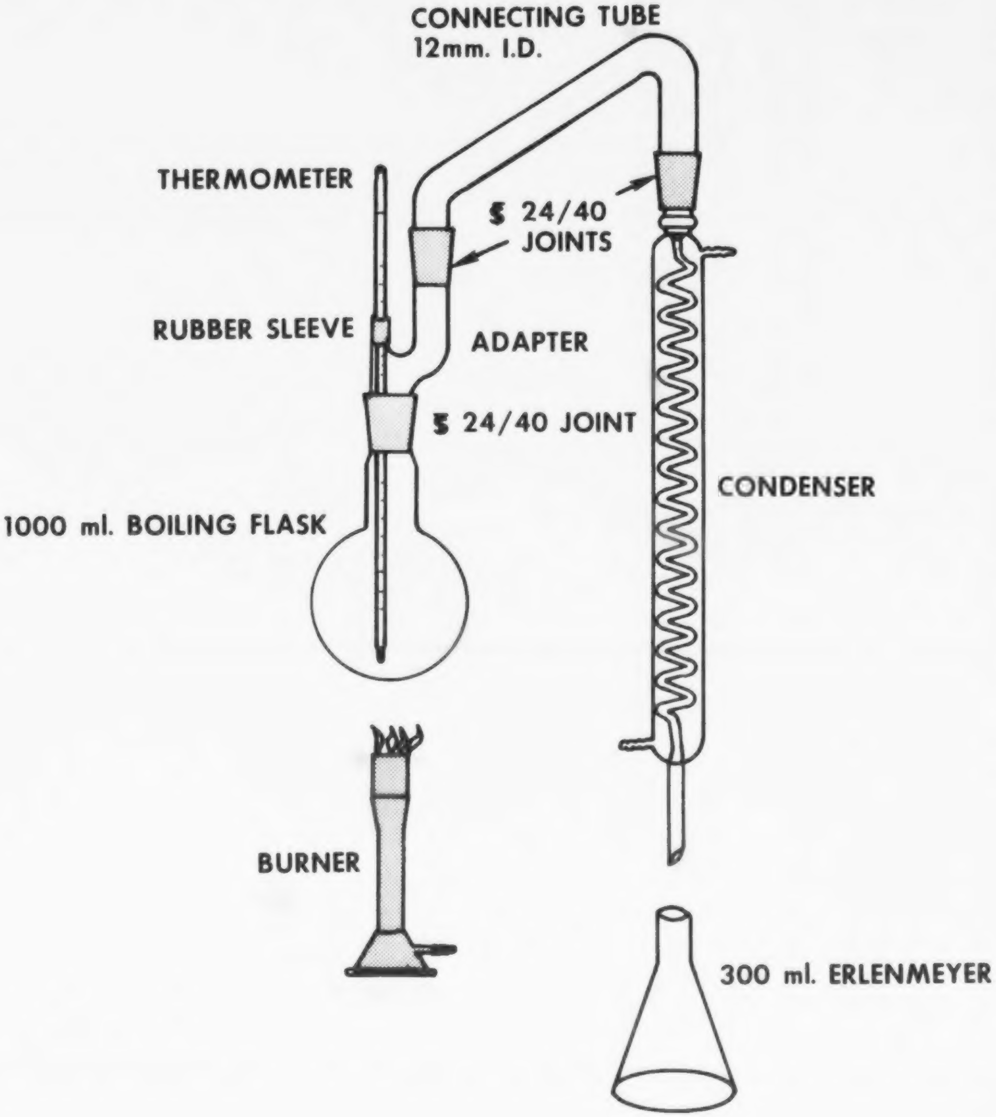

CONNECTING TUBE
12mm. I.D.
THERMOMETER
$ 24/40
JOINTS
RUBBER SLEEVE
ADAPTER
$ 24/40 JOINT
CONDENSER
1000 ml. BOILING FLASK
BURNER
300 ml. ERLENMEYER

FIGURE 1. DISTILLATION APPARATUS

WATER MONITORING PROCEDURE: Preliminary Distillation Procedure for Fluoride Analysis of Potable and Wastewaters

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INTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	The Guidelines Establishing Test Procedures for the Analysis of Pollutants lists three approved analytical methods for fluoride. It is mandatory to precede each by the preliminary distillation procedure, unless comparability data is available on a representative effluent sample to show that this preliminary distillation is not necessary.	Federal Register, Part II, Wednesday, Dec. 1, 1976, page 52782, Parameter 20.
	The National Interim Primary Drinking Water Regulations lists two approved analytical methods for fluoride. It is mandatory to precede only the colorimetric (SPADNS) method by the preliminary distillation procedure.	Federal Register, Part IV, Wednesday, Dec. 24, 1975, page 59573, Parameter 10.
	The Preliminary Distillation step required to be used is the method utilizing a distillation from sulfuric acid.	Standard Methods for the Examination of Water and Wastewater. 13th ed., page 171. 14th ed., page 389.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.2.4	Care should be taken to assure complete mixing between the acid and water or sample. Incomplete mixing will result in a violent bumping of the still which can throw the acid mixture out of the distilling flask and possible damage to the still, analyst and loss of sample.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF BARIUM (Ba^{++})

as applied in

WATER AND WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

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WATER MONITORING PROCEDURE: Determination of Barium (BA^{++})

1. Analysis Objectives:

   The learner will be instructed on how to determine the barium content of a sample.

2. Brief Description of Analysis:

   The sample is first digested with acid to assure all metals are in a soluable form. After which a portion of the sample is aspirated into an atomic absorption spectrophotometer utilizing an acetylene-nitrous oxide flame.

3. Applicability of the Procedure:

   The method works for both potable and wastewaters.

   a. Range of Concentration - The method is recommended for use in the range of 1.0 to 20 mg of barium/liter. The detection limit is 0.03 mg/l.

   b. Pretreatment of the sample - Digestion in acid pH to assure solubilization. See Section B.

   c. Treatment of Interferences in the sample - The use of the nitrous oxide-acetylene flame virtually eliminates chemical interferences; however, barium is easily ionized in this flame and potassium must be added to standards and samples alike to control this effect.

   d. Source of Procedure - Manual of Methods for Chemical Analysis of Water and Wastes; 1974 ed., p. 97; USEPA Technology Transfer, Cinti., OH 45268.

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WATER MONITORING PROCEDURE: Determination of Barium (BA^{++})

Operating Procedures:

A. Equipment Preparation

B. Reagent Preparation

C. Standard Preparation

D. Sample Pretreatment

E. Instrument Calibration

F. Sample Analysis

G. Calculations

WATER MONITORING PROCEDURE: Determination of Barium (BA^{++})

Equipment and Supply Requirements

A. Capital Equipment:

1. Atomic absorption spectrophotometer
2. Recorder - compatible with the instrument
3. Nitrous oxide burner head
4. Barium - hollow cathode lamp
5. Pressure regulators two stage for
   Acetylene - CGA inlet 510 connector
   Air - CGA inlet 1340 connector
   Nitrous oxide - CGA inlet 1320 connector
6. Balance - analytical with a 0.1 milligram sensitivity
7. Still - borosilicate glass or equivalent

B. Reusable Supplies:

1. Trip balance - 100 gram capacity
2. Pen or pencil
3. Twelve inch ruler
4. Hot plate
5. Five reagent bottles - clear glass, glass stoppered 500 ml cap
6. Six beakers - glass, 150 ml size
7. Six cylinders - graduated, 100 ml size
8. Three flasks - volumetric, 1000 ml volume
9. Six flasks - volumetric, 100 ml volume
10. One pipet - graduated, 1 ml
11. Two pipets - graduated, 10 ml
12. One pipet - volumetric, 1 ml
13. Two pipets - volumetric 5 ml
14. One pipet - volumetric 10 ml
15. One pipet - volumetric 20 ml
16. One funnel - powder, glass
17. One funnel - filtering
18. Pipet bulb
19. Safety glasses
20. Wash bottle - plastic

Consumable Supplies:

1. Deionizing column - mixed bed type
2. Gases
   Acetylene - purified or commercial grade
   Air - dry grade
   Nitrous oxide - technical grade
3. Detergent
4. Nitric Acid - ACS grade
5. Hydrochloric acid - ACS grade
6. Barium chloride
7. Potassium chloride

WATER MONITORING PROCEDURE: Determination of Barium (BA^{++})

Equipment and Supply Requirements (Continued)

8. pH paper - capable of measuring pH 2 (pHydrion)
9. Filter paper - Whatman #42
10. Graph paper
11. Wax marking pencil
12. Plastic weighing boats, ∿ 12 each

WATER MONITORING PROCEDURE: Determination of Barium (Ba^{++})

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Cleaning of Glassware	1. Wash with detergent.	1a. Cleaning should be carried out in this order. 1b. All glassware should be kept covered after cleaning.	
	2. Rinse with tap water.		
	3. Rinse with 1:1 nitric acid.	3a. Add an equal volume of acid to an equal volume of distilled water. (Example: 500 ml of acid added to 500 ml of water. 3b. Caution: Always add acid to water not the reverse.	
	4. Rinse with tap water.		
	5. Rinse with 1:1 hydrochloric acid.	5a. Add equal volume of HCl to an equal volume of distilled water.	
	6. Rinse with tap water.		
	7. Rinse with distilled water.		
2. Balance Inspection	1. Check all balances for cleanliness and proper operation.	1a. Consult the manufacturer's manual if the balance does not operate properly.	
3. Instrument Inspection	1. Check all items of the instrument to assure proper optimization and operation.	1a. Consult the manufacturer's manual.	

WATER MONITORING PROCEDURE: Determination of Barium (Ba^{++})

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation			
1. Distilled Deionized Water	1. Prepare by passing distilled water through a mixed bed of cation and anion exchange resins.	1a. Use deionized distilled water for the preparation of all reagents, calibration standards and as dilution water.	
2. Nitric Acid Concentrated (HNO_3)	1. Commercially available reagent grade.	1a. If a high reagent blank is obtained, it may be necessary to distill the acid or purchase a better purity.	
3. Nitric Acid 1:1	1. Prepare a 1:1 solution from reagent grade nitric acid by adding an equal amount of the acid to an equal amount of distilled water.	1a. Caution: add the acid to water; not the reverse.	
4. Hydrochloric Acid Concentrated (HCl)	1. Commercially available reagent grade.		
5. Hydrochloric Acid 1:1	1. Prepare a 1:1 solution from reagent grade hydrochloric acid by adding an equal amount of the acid to an equal amount of distilled water.	1a. Caution: add the acid to water; not the reverse.	
6. Barium Stock Solution	1. Carefully weigh 1.7787 grams of barium chloride ($BaCl_2 \cdot 2H_2O$) (analytical reagent grade).	1a. Use a plastic weighing boat and an analytical balance.	
	2. Transfer into a 1000 ml volumetric flask.	2a. Use a powder funnel. 2b. Use a plastic wash bottle to rinse weighing boat and funnel into the flask.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	3. Dissolve in distilled water.		
	4. Dilute to the mark with distilled water.	4a. One ml equals 1 mg Ba (1000 mg/liter)	
7. Barium Standard Solution	1. Transfer about 50 ml of distilled deionized water to a 100 ml volumetric flask.	1a. This volume need not be measured exactly.	
	2. Pipet 1 ml of barium stock solution (Reagent 6) into the flask.	2a. Use a 1 ml volumetric pipet.	
	3. Dilute to the mark with distilled deionized water.	3a. This solution contains 10 mg Ba/liter. (1 ml = 0.01 mn Ba)	
8. Potassium Chloride Solution (KCl)	1. Weigh 95 grams of potassium chloride (KCl) (analytical reagent grade).	1a. Use a plastic weighing boat on a trip balance.	
	2. Transfer into a 1000 ml volumetric flask.	2a. Use a powder funnel. 2b. Use a plastic wash bottle to rinse the weighing boat and funnel into flask.	
	3. Dissolve in distilled water.		
	4. Dilute to the mark with distilled water.	4a. One ml = 50 mg K = (50,000 mg/liter).	
9. Fuel and Oxidant	1. Commercial grade acetylene is generally acceptable.		

WATER MONITORING PROCEDURE: Determination of Barium (Ba^{++})

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	2. Reagent grade nitrous oxide (N_2O) is required.		
	3. Air - dry grade.	3a. Air may be supplied from a compressed air line, a compressor or cylinder. 3b. Air is used to ignite the burner initially on most instruments, then the flame is switched over to the nitrous oxide. This reduces the possibility of flash back occurring. 3c. Caution: The air supply must be free from oil or other contaminants.	
C. Standard Preparation	1. Collect 5 clean 100 ml volumetric flasks.		
	2. Add about 50 ml deionized distilled water to each flask.	2a. This volume is not critical--just fill flask about half full.	
	3. Add 0.2 ml concentrated nitric acid.	3a. The calibration standards should be prepared using the same type of acids (HCl and HNO_3) and at the same concentrations as the samples for analysis. 3b. Nitric acid will have been added to the sample for preservation. 3c. Use a 1 ml pipet graduated in tenths. 3d. Use caution when pipetting concentrated acids. 3e. Use a pipet bulb and safety glasses.	
	4. Add 5.0 ml of concentrated hydrochloric acid.	4a. Use a 10 ml graduated pipet.	
	5. Add 2.0 ml of the potassium chloride solution (Reagent 8)	5a. Use a 10 ml graduated pipet.	VI.C.5 (p. 17)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standard Preparation (Continued)	6. Transfer 0.0, 5.0, 10.0, 15.0, 20.0 ml of the barium standard solution (Reagent 6) to each of the five volumetric flasks respectively.	6a. Use volumetric pipets for the transfer. 6b. The blank (the 0.0 flask) will not need to be pipetted.	
	7. Dilute each flask to the volume mark with deionized distilled water.	7a. The solutions contain 0.0, 0.5, 1.0, 1.5, and 2.0 mg Ba/liter of solution.	
	8. Mix thoroughly.		
D. Sample Pretreatment: for Total Metal	1. Transfer 100 ml of sample into a clean 150 ml beaker.	1a. Use a 100 ml graduated cylinder.	
	2. Check the pH.	2a. pH paper can be used (pHydrion). 2b. pH must be <pH2.	
	3. Adjust pH to below 2 if it has not been adjusted during sampling.	3a. Add concentrated nitric acid (HNO_3).	
	4. Add 5 ml of 1:1 hydrochloric acid.	4a. Use a 5 ml volumetric pipet.	
	5. Heat the sample at 95°C for 15 minutes.	5a. On a hot plate or water bath. 5b. The sample should not boil.	
	6. Cool to room temperature.		
	7. Wash down walls of the beaker with distilled water.	7a. Use a plastic wash bottle. 7b. The final volume is to be 100 ml. Do not use large amount of water.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Sample Pretreatment: for Total Metal (Continued)	8. Filter the sample through a filter paper into a 100 ml graduated cylinder.	8a. A paper such as Whatman #42 should be used.	
	9. Adjust the volume to 100 ml.	9a. The sample is now ready for analysis.	
E. Instrument Calibration	1. Install the nitrous oxide burner head.	1a. Follow the manufacturer's directions for installation. Usually no tools are required and the installation is not difficult.	
	2. Assure that the barium lamp is in the light path.	2a. Depending on the instrument, the analyst may have to place the lamp into the lamp compartment or revolve the turret.	
	3. Adjust the wavelength to the proper setting.	3a. 553.6 nm for barium.	
	4. Select the proper slit arrangement.	4a. Consult the manufacturer's specifications or procedures manual.	
	5. Set the lamp current control to its lowest position.	5a. Consult the manual for location on your instrument.	
	6. Turn on the power to the instrument.	6a. Consult the manufacturer's manual for location. 6b. Allow warm up time for single beam instruments.	
	7. Adjust the lamp current control until the proper milliamps are applied to the lamp.	7a. Consult the lamp for proper operating current.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Instrument Calibration (Continued)	8. Turn on the master valves for the air, acetylene and nitrous oxide.	8a. The pressure setting for the gases should be set to the manufacturer's recommendations.	
	9. Ignite flame.	9a. Using air-acetylene: after ignition, switch to nitrous oxide. 9b. Adjust the acetylene for maximum absorption. 9c. See instruction manual for your particular instrument.	
	10. Aspirate the standard solution prepared in Section C.	10a. Record the response on a recorder or its equivalent.	
	11. Prepare a calibration curve by plotting the concentration, in mg Ba/liter, of the standards against the response for each concentration.		
F. Sample Analysis	1. Each sample must be pretreated to assure that all materials which might contain barium are in soluble form.	1a. Steps are in Section C.	
	2. Add 2 ml of the potassium chloride solution (Reagent 8).	2a. Use a 10 ml graduated pipet.	
	3. Aspirate the unknown solution(s) into the instrument immediately following the aspiration of the standards.	3a. The standard curve produced under Section E must be verified each time barium is to be analyzed. 3b. The flame characteristics and instrument settings should be the same for standards and unknowns.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Analysis (Continued)	4. Record the response.		
G. Calculations	1. Determine the concentration of barium in the sample by substituting the observed instrumental response on the calibration graph. 2. Express all values as mg Ba/liter.	1a. Consult the outline in Section F concerning "Calibration Graphs." 1b. Some instruments can be calibrated to read directly in concentration. This should be used only after the analyst is assured that correct responses can be attained.	

WATER MONITORING PROCEDURE: Determination of Barium (Ba^{++})

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	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.4.1a	As acetylene (C_2H_2) is packed dissolved in acetone (CH_3COCH_3), cylinders should be stored only in an upright position. Acetone can be introduced into the flame if the cylinder has been stored lying on its side or if used below 75 psig. Acetone entrainment usually produces a slight pink tinge and abnormally high background signals.	
B.9.2	The use of a nitrous oxide-acetylene flame is recommended for the determination of barium. This flame virtually eliminates chemical interferences. However, if nitrous oxide is not available an air-acetylene flame can be used.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.8	Barium is easily ionized in the nitrous oxide flame. Ionization will produce incorrect results. In order to suppress the ionization effect potassium is added to standard and sample alike. The concentration in the standards and samples should equal 1000 mg K/1000 ml of solution. For the concentration used in the preparation, 2 ml of the potassium solution should be added per 100 ml of standard or sample.	
B.7 C.5	If the nitrous oxide flame is not availabe and the acetylene-air flame is used, phosphate, silicon and aluminum will severely depress the barium absorbance. This may be overcome by the addition of 2000 mg La/liter. This solution is prepared as follows: Dissolve 58.65 g lanthanum oxide (La_2O_3) in 250 ml of concentrated hydrochloric acid (Caution: reaction is violent; use a hood). Dilute to 500 ml volume. This solution contains 100 mg La/ml. Add 2 ml of this solution to each 100 ml of standard and/or sample. This gives a final concentration of 2000 mg La/1000 ml of solution.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.3a	In order to comply with the quality control section of the "Criteria and Procedures for Water Supply Laboratory Certification," the minimum requirement is to run a validation of the standard curve consisting of at least a reagent blank and one standard at or near the maximum contaminant level of 1.0 mg Ba/liter. This should be done daily or with each batch of samples. If 20 or more samples are run daily, the standard curve must be verified by running an additional standard of midrange every 20 samples. All checks must be within ± 10 percent of the original curve. If not, a new standard curve must be prepared.	Criteria and Procedures for Water Supply Laboratory Certification. Office of Research and Development, U.S. EPA, Washington, DC 20460.
	The control recommended for water pollution analysis as listed in the Handbook for Analytical Quality Control in Water and Wastewater Laboratories is to verify the standard curve by two standards, one high and one low concentration.	Handbook for Analytical Control in Water and Wastewater Laboratories. U.S. EPA Technology Transfer, Cinti., OH 45268.

RESIDUAL CHLORINE AND TURBIDITY

I. INTRODUCTION

The Interim Primary Drinking Water Regulations (Federal Register, December 24, 1975) permits the options of substitution of up to 75 percent of the bacteriological samples with residual chlorine determinations. Any community or non-community water system may avail themselves of this option with approval from the State based upon results of sanitary surveys. Residual chlorine determinations must be carried out at the frequency of at least four for each substituted microbiological sample.

Since many potable water plants carry out their own microbiological determinations, it will be necessary that these laboratories be certified for the bacteriological parameters. Residual chlorine determinations may be carried out by any person acceptable to the State and the analytical method and techniques used must be evaluated in some manner to assure that reliable information is obtained.

Since the presence of high turbidity can interfere with the disinfection capability of chlorine, a maximum allowable limit has been set for turbidity as follows:

A. One turbidity unit (TU) as determined by a monthly average except that five or fewer turbidity units may be allowed if the supplier of water can demonstrate to the State that the higher turbidity does not

   1. Interfere with disinfection,

   2. Prevent maintenance of residual of disinfectant throughout distribution system, or,

   3. Interfere with microbiological determinations.

B. Five turbidity units based on an average of two consecutive days.

   The Criteria and Procedures Document for Water Supply Laboratory Certification suggests that some quality control guidelines be instituted for the residual chlorine and turbidity measurements at the State level for the purpose of ensuring data validity for these critical measurements.

   In response to public comments regarding the proposed Primary Regulations (Federal Register, December 24, 1975) it is stated that operators performing residual chlorine and turbidity analyses "....be certified, approved, or at least minimally trained to perform the analytical tasks before a State could accept their analytical determinations...."

II. RESIDUAL CHLORINE

Since residual chlorine analysis would be carried out in "field" conditions or in the small laboratories of treatment plants, perhaps by unskilled operators, it is necessary to keep the analytical method as simple as possible. For a number of years, operators had utilized the orthotolidine technique in a kit form to determine the chlorine residual. Recent studies and regulatory guidelines have dictated against this test procedure. The acceptable test procedure is now the DPD Test (13th Ed., Standard Methods for the Examination of Water and Wastewater, pgs. 129-132), for which kits are available from at least two companies and which meet requirements for accuracy and reliability. These kits are capable of measuring both free and combined chlorine of which only the free chlorine is measured to meet compliance requirements. Kit procedures call for a premeasured single powder or tablet reagent added to the test cell with the sample and a resultant color development measures by comparison the standardized colors within one minute. Standard Methods includes cautions regarding temperature and pH control regarding this test parameter and this test procedure, the DPD Test, is least effected by temperature and the pH is adjusted by the added reagents. The only interfering substance, oxidized manganese, can be determined in a preliminary step and compensated for in the final test value.

III. TURBIDITY

Turbidity has long been used in the water supply industry for indicating proper operational techniques. Turbidity should be clearly understood to be an expression of the optical property of a sample which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample.

The standard method for the determination of turbidity has been based on the Jackson candle turbidimeter. However, the lowest turbidity value which can be measured directly on the Jackson turbidimeter is 25 units which is well above the monitoring level. Because of these low level requirements, the nephelometric method was chosen and procedures are given in Standard Methods (13th Ed., 1971).

IV. NEPHELOMETRIC MEASUREMENTS FOR COMPLIANCE MONITORING

The subjectivity and apparatus deficiencies involved in visual methods of measuring turbidity make each unsuitable as a standard method.

Since turbidity is an expression of the optical property of scattering or absorbing light, it was natural that optical instruments with photometers would be developed for this measurement.

The type of equipment specified for compliance monitoring(3,6) utilizes nephelometry.

A. Basic Principle(7)

The intensity of light scattered by the sample is compared (under defined conditions) with the intensity of light scattered by a standard reference solution (formazin). The greater the intensity of scattered light, the greater the turbidity. Readings are made and reported in NTUs (Nephelometric Turbidity Units).

B. Schematic

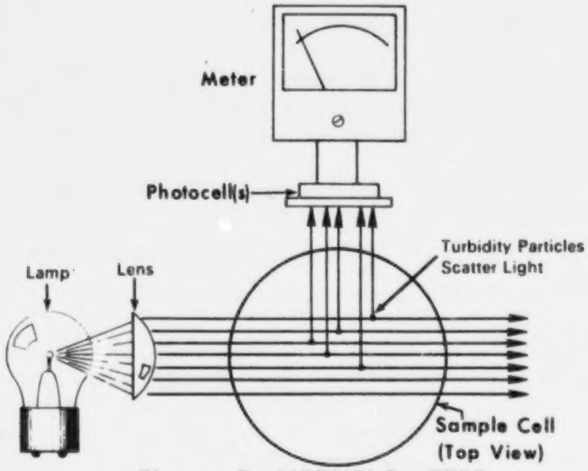

Figure 2 NEPHELOMETER
(90° Scatter)

Light passes through a polarizing lens and on to the sample in a cell. Suspended particles (turbidity) in the sample scatter the light.

Photocell(s) detect light scattered by the particles at a 90° angle to the path of the incident light. This light energy is converted to an electric signal for the meter to measure.

1. Direction of Entry of Incident Light to Cell

   a. The lamp might be positioned as shown in the schematic so the beam enters a sample horizontally.

   b. Another instrument design has the light beam entering the sample (in a flat-bottom cell) in a vertical direction with the photocell positioned accordingly at a 90° angle to the path of incident light

2. Number of Photocells

   The schematic shows the photocell(s) at one 90° angle to the path of the incident light. An instrument might utilize more than one photocell position, with each final position being at a 90° angle to the sample liquid.

3. Meter Systems

   a. The meter might measure the signal from the scattered light intensity only.

   b. The meter might measure the signal from a ratio of the scattered light versus light transmitted directly through the sample to a photocell.

4. Meter Scales and Calibration

   a. The meter may already be calibrated in NTUs. In this case, at least one standard is run in each instrument range to be used in order to check the accuracy of the calibration scales.

   b. If a pre-calibrated scale is not supplied, a calibration curve is prepared for each range of the instrument by using appropriate dilutions of the standard turbidity suspension.

C. EPA Specifications for Instrument Design(7)

Even when the same suspension is used for calibration of different nephelometers, differences in physical design of the turbidimeters will cause differences in measured values for the turbidity of the same sample. To minimize such differences, the following design variables have been specified by the U. S. Environmental Protection Agency.

1. Defined Specifications

   a. Light Source

   Tungsten lamp operated at not less than 85% of rated voltage and at not more than rated voltage.

   b. Distance Traveled by Light

   The total of the distance traversed by the incident light plus scattered light within the sample tube should not exceed 10 cm.

   c. Angle of Light Acceptance of the Detector

   Detector centered at 90° to the incident light path and not to exceed ± 30° from 90°.

   (Ninety degree scatter is specified because the amount of scatter varies with size of particles at different scatter angles).

   d. Applicable Range

   The maximum turbidity to be measured is 40 units. Several ranges will be necessary to obtain adequate coverage. Use dilution for samples if their turbidity exceeds 40 units.

2. Other EPA Design Specifications

   a. Stray Light

   Minimal stray light should reach the photocell(s) in the absence of turbidity.

Some causes of stray light reaching the photocell(s) are:

1) Scratches or imperfections in glass cell windows.
2) Dirt, film or condensation on the glass.
3) Light leakages in the instrument system.

A schematic of these causes is shown in Figure 3.

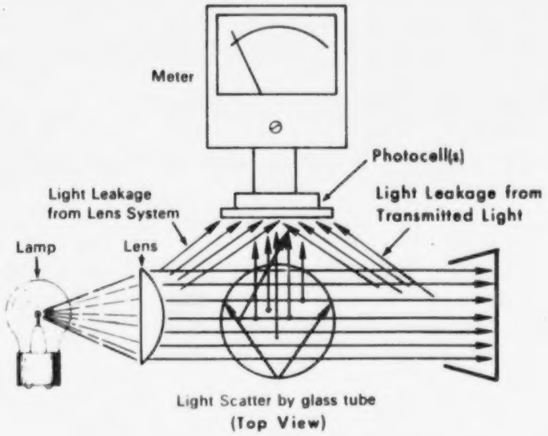
Meter
Photocell(s)
Light Leakage from Lens System
Light Leakage from Transmitted Light
Lamp
Lens
Light Scatter by glass tube
(Top View)

Figure 3 NEPHELOMETER SOURCES OF STRAY LIGHT

Stray light error can be as much as 0.5 NTU. Remedies are close inspection of sample cells for imperfections and dirt, and good design which can minimize the effect of stray light by controlling the angle at which it reaches the sample.

b. Drift

The turbidimeter should be free from significant drift after a short warm-up period. This is imperative if the analyst is relying on a manufacturer's solid scattering standard for setting overall instrument sensitivity for all ranges.

c. Sensitivity

In waters having turbidities less than one unit, the instrument should detect turbidity differences of 0.02 unit or less. Several ranges will be necessary to obtain sufficient sensitivity for low turbidities.

3. Examples of instruments meeting the specifications listed in 1 and 2 above include:

   a. Hach Turbidimeter Model 2100 and 2100A.

   b. Hydroflow Instruments DRT 100, 200, and 1000.

4. Other turbidimeters meeting the listed specifications are also acceptable.

D. Sources of Error

1. Sample Cells

   a. Discard scratched or etched cells.

   b. Do not touch cells where light strikes them in instrument.

   c. Keep cells scrupulously clean, inside and out.(8)

      1) Use detergent solution.

      2) Organic solvents may also be used.

      3) Use deionized water rinses.

      4) Rinse and dry with alcohol or acetone.

2. Standardizing Suspensions(7)

   a. Use turbidity - free water for preparations. Filter distilled water through a 0.45μm pore size membrane filter if such filtered water shows a lower turbidity than the distilled water.

   b. Prepare a new stock suspension of Formazin each month.

   c. Prepare a new standard suspension and dilutions of Formazin each week.

3. Sample Interferences

   a. Positive

      1) Finely divided air bubbles

   b. Negative

      1) Floating debris

      2) Coarse sediments (settle)

      3) Colored dissolved substances (absorb light)

E. Reporting Results(7)

NTU	RECORD TO NEAREST
0.0-1.0	0.05
1-10	0.1
10-40	1
40-100	5
100-400	10
400-1000	50
>1000	100

F. Precision and Accuracy(7)

1. In a single laboratory (EMSL), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard divi[illegible]ons were ±0.60, ±0.94, ±1.2 and ±4.7 units, respectively.

2. Accuracy data is not available at this time.

V. STANDARD SUSPENSIONS AND RELATED UNITS(9)

One of the critical problems in measuring turbidity has been to find a material which can be made into a reproducible suspension with uniform sized particles. Various materials have been used.

A. Natural Materials

1. Diatomaceous earth
2. Fuller's earth
3. Kaolin
4. Naturally turbid waters.

Such suspensions are not suitable as reproducible standards because there is no way to control the size of the suspended particles.

B. Other materials

1. Ground glass
2. Microorganisms
3. Barium Sulfate
4. Lates spheres

Suspensions of these also proved inadequate.

C. Formazin

1. A polymer formed by reacting hydrazine sulfate and hexamethylenetetramine sulfate.

2. It is more reproducible than previously used standards. Accuracy of ± one percent for replicate solutions has been reported.

3. In 1958, the Association of Analytical Chemists initiated a standardized system of turbidity measurements for the brewing industry by:

   a. Defining a standard formula for making stock Formazin solutions and

   b. Designating a unit of measurement based on Formazin, i.e., the Formazin Turbidity Unit (FTU).

4. During the 1960's Formazin was increasingly used for water quality turbidity testing. It is the currently recognized standard for compliance turbidity measurements.

D. Units

1. At first results were translated into Jackson Turbidity Units (JTU). However, the JTU was derived from a visual measurement using concentrations (mg/liter) of silica suspensions prepared by Jackson. They have no direct relationship to the intensity of light scattered at 90 degrees in a nephelometer.

2. For a few years, results of nephelometric measurements using specified Formazin standards were reported directly as Turbidity Units (TUs).

3. Currently, the unit used is named according to the instrument used for measuring turbidity. Specified Formazin standards are used to calibrate the instrument and results are reported as Nephelometric Turbidity Units (NTUs).

VI. SUMMARY

The importance of residual chlorine determination can be seen in its possible effect on the health of the consumers. The Criteria and Procedures for Laboratory Certification suggests that some form of quality assurance should be instituted on a state level to assure valid data for both the chlorine and turbidity measurements. The comments on the public responses to the proposed Interim Primary Regulations also suggests some form of quality assurance on the state level to be instituted. Consequently, the Regional Certification team should point out to the principal laboratories the importance of some kind of effort being instituted. States might wish to offer some kind of formal training effort as part of the approval mechanism for the operators doing the chlorine and/or turbidity measurements.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

MEASUREMENT OF FREE CHLORINE UTILIZING THE DPD KIT

as applied in

DRINKING WATER TREATMENT FACILITIES
and in the
DISTRIBUTION SYSTEMS OF DRINKING WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

1. Analysis Objectives:

The actual use of a kit form of the DPD method for chlorine is so simple a procedure, it need not be written. However, the permanent standards, whether liquid or solid, must be calibrated. This unit sets down a method that can be used to accomplish this. The method is applicable to drinking waters only for compliance purposes.

2. Brief Description of Analysis:

The kit usually contains a comparator which is a holder of sorts to support the sample cell and the standard in such manner as to allow the operator to see both colors and compare their intensities. The operator adds a single reagent to the water to be analyzed. The color is formed immediately and a comparison is made between the sample and standards.

3. Applicability of this Procedure:

   a. Range of concentration - 0.0 to about 3.0 mg chlorine/liter or whatever is the highest standard supplied with the kit.
   b. Pretreatment - the sample may not be preserved; it should be run as soon as possible or within one hour after being taken.
   c. Treatment of Interferences in Samples: The procedure includes instructions for the determination of the interference caused if oxidized manganes is present. Other interferences are suppressed by the make-up of the reagents.

Source of this procedure: Standard Methods for the Examination of Water and Wastewater, 13th ed., pp. 129-132 or 14th ed. pp. 329-334.

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WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

Equipment and Supply Requirements

A. Capital Equipment:

Analytical balance capable of weighing to 0.1 mg (.0001 g) under a 200 g load.

B. Reusable Supplies:

1. Chlorine Test Kit (DPD method) such as:
   a. Model CN-66
      Hach Chemical Company
      PO Box 907
      Ames, IA 50010

   b. Model LP-1
      LaMotte Chemical Products Company
      Chestertown, MD 21620
2. Eight beakers, 50 ml size
3. Two flasks, volumetric with stoppers, 1000 ml size
4. Ten flasks, volumetric with stoppers, 100 ml size
5. One pipet, volumetric, 100 ml size
6. One pipet, volumetric, 50 ml size
7. One pipet, volumetric, 20 ml size
8. Three pipets, volumetric, 10 ml size
9. One dropper bottle, 100 ml size
10. Two rubber stoppers - to fit comparator cells (if stoppers are not supplied by manufacturer)

C. Consumable Supplies:

1. Distilled water, about 1 gal. or 3.8 liters
2. Graph paper, arithmetic, 10 x 10 divisions
3. Pencil or pen
4. Wash bottle, plastic squeeze type
5. Weighing boat, plastic, disposable
6. Potassium Permanganate ($KMnO_4$)
7. Potassium Iodide Crystals (KI)
8. Sodium Arsenite ($NaAsO_2$)
9. DPD - Reagent (Usually a supply is provided with the kit.)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Reagent Preparation			
1. Stock Potassium Permanganate Solution	1. Weigh out 0.891 grams of potassium permanganate ($KMNO_4$).	1a. Use an analytical balance. 1b. Use a plastic weighing boat.	
	2. Transfer the potassium permanganate to a 1000 ml volumetric flask.		
	3. Wash the weighing boat	3a. With distilled water. 3b. Use a plastic squeeze type wash bottle. 3c. The wash should be about 10 to 20 ml.	
	4. Pour the washing into the volumetric flask.		
	5. Repeat washing two more times.	5a. The idea is to assure complete transfer of the potassium permanganate into the volumetric flask. Since potassium permanganate has a strong color, wash until no color is seen in the weighing boat containing water.	
	6. Add enough water to fill the flask about half full.		
	7. Swirl until the potassium permanganate is dissolved.	7a. If a magnetic stirrer is available, this can be used to save time.	
	8. Dilute to the mark on the volumetric flask.	8a. There is a single ring etched around the neck of the flask. When the flask is filled to this mark, it will contain the volume stated on the flask.	
	9. Stopper and mix by repeated inversions.		

WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Reagent Preparation (Continued)			
2. Intermediate Potassium Permanganate Solution	1. Transfer 10.00 ml of the stock potassium permanganate solution into a 100 ml flask.	1a. Use a 10.0 ml volumetric pipet.	
	2. Dilute to the mark with distilled water.	2a. Add water from a plastic squeeze bottle.	
	3. Stopper and mix by repeated inversions.		
3. Standard Potassium Permanganate Solution	1. Transfer 10.00 ml of the intermediate potassium permanganate solution to a 1000 ml volumetric flask.		
	2. Add distilled water to the flask to the mark.		
	3. Stopper and mix by repeated inversions.	3a. This solution has a chlorine equivalent of 1.00 mg/l in the DPD reaction.	
4. Potassium Iodide Crystals	1. No preparation necessary.		
5. Sodium Arsenite ($NaAsO_2$)	1. Weigh out 0.500 grams of sodium arsenite.	1a. Use a trip balance or an analytical balance. 1b. Use a plastic weighing boat. 1c. CAUTION: TOXIC - take care to avoid injestion.	
	2. Transfer the sodium arsenite to a 100 ml volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Reagent Preparation (Continued)	3. Rinse the weighing boat.	3a. With distilled water. 3b. Use a plastic squeeze bottle.	
	4. Add the rinses to the volumetric flask.		
	5. Add enough distilled water to fill the flask about half full.		
	6. Swirl until the sodium arsenite is dissolved.		
	7. Dilute to the mark on the volumetric flask.	7a. There is a single ring etched around the neck of the flask. When the flask is filled to this mark, it will contain the volume stated on the flask.	
	8. Stopper and mix by repeated inversion.		
	9. Transfer to a dropper bottle, and label.	9a. Label "Sodium Arsenite."	
6. Preparation of the Standard Series	1. Prepare a series of dilutions of the standard potassium permanganate solution in 100 ml volumetric flasks.	1a. The series should contain several concentrations below and above the expected values. 1b. The expected value for drinking water would be near 0.2 mg/l of free chlorine. 1c. The series can be prepared as follows:	

WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>A. Reagent Preparation (Continued)</td><td></td><td>
<table>
<tr><th>Col. 1</th><th>Col. 2</th><th>Col. 3</th><th colspan="2">Col. 4</th><th>Col. 5</th></tr>
<tr><th>ml of Std. $KMnO_4$</th><th>ml of Water</th><th>Conc. Cl^- mg/l</th><th colspan="2">Comparator Values mg Cl^-/l</th><th>Avg. Value $\frac{a + b}{2}$ mg/l</th></tr>
<tr><th></th><th></th><th></th><th>a</th><th>b</th><th></th></tr>
<tr><td>0</td><td>100</td><td>0.0</td><td></td><td></td><td></td></tr>
<tr><td>10</td><td>90</td><td>0.1</td><td></td><td></td><td></td></tr>
<tr><td>20</td><td>80</td><td>0.2</td><td></td><td></td><td></td></tr>
<tr><td>30</td><td>70</td><td>0.3</td><td></td><td></td><td></td></tr>
<tr><td>40</td><td>60</td><td>0.4</td><td></td><td></td><td></td></tr>
<tr><td>50</td><td>50</td><td>0.5</td><td></td><td></td><td></td></tr>
<tr><td>70</td><td>30</td><td>0.7</td><td></td><td></td><td></td></tr>
<tr><td>100</td><td>0</td><td>1.0</td><td></td><td></td><td></td></tr>
</table>
</td><td></td></tr>
<tr><td>B. Calibration of the Kit Standards</td><td>1. Fill the reference tube with distilled water.</td><td>1a. Not all kits have a reference tube.
1b. Follow the manufacturer's directions for performing a test with their comparator.</td><td></td></tr>
<tr><td></td><td>2. Rinse the sample cell with the water to be tested.</td><td>2a. In this case, one of the standards.
2b. Read from low to high on the series.</td><td></td></tr>
<tr><td></td><td>3. Fill the cell with the water to be tested to the mark.</td><td>3a. Most kits have a line around the tube. This indicates a calibrated volume. The cells should always be filled to this mark.</td><td></td></tr>
<tr><td></td><td>4. Add the manufacturer's DPD reagent.</td><td>4a. This is usually in the form of a powder or a tablet.
4b. This reagent contains both the DPD indicator and the buffer as prescribed in the DPD method in the "Standard Methods" references.</td><td></td></tr>
</table>

WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Calibration of the Kit Standards	5. Stopper the cells.	5a. Most manufacturers provide stoppers. If not, procure two rubber stoppers. The diameter of the tubuler cells varies so check the size needed.	
	6. Mix by inversion.	6a. One inversion will do.	
	7. Place cell in comparator.		
	8. Hold comparator up to the light.	8a. Rotate disc and/or compare color in tube with the standard colors.	
	9. Obtain a match between color in cell and standard.	9a. Where the comparator has only specific values such as 0.2, 0.4, 0.6, etc. If the color is not an exact match the mid-point value between the two closest colors is used.	
	10. Record the value obtained from the comparator.	10a. Along with the known concentration	
	11. Discard the solution.		
	12. Rinse the cell with the next standard to be run.		
	13. Discard.		
	14. Fill to the line with the next standard.		
	15. Repeat steps 4 through 11 for all standards.		
	16. Rinse the tube with the first standard read.	16a. The entire series should be read again to obtain a second value. 16b. The values should be recorded in Column 4 of Table A.6.1c.	

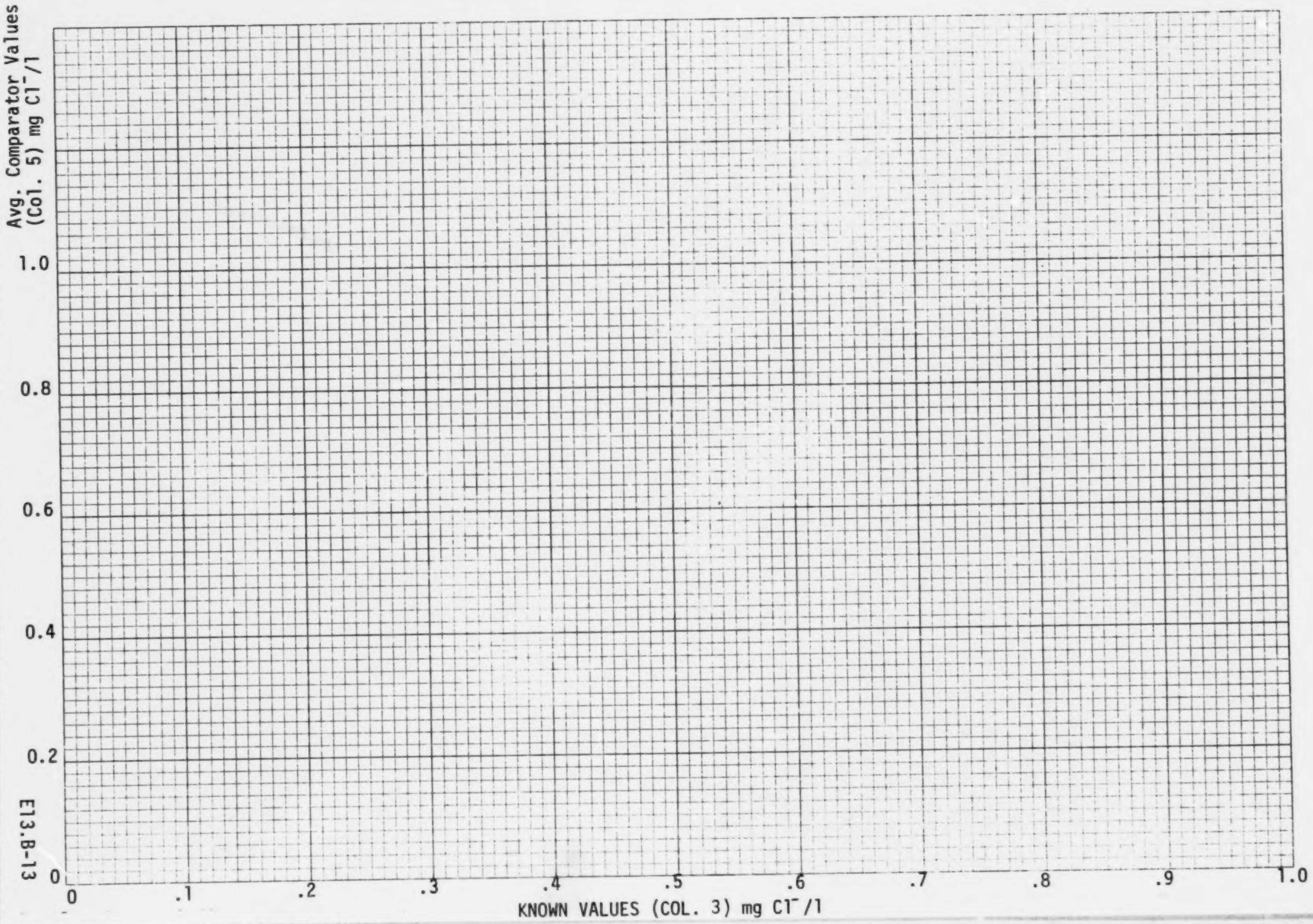
WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Calibration of the Kit Standards (Continued)	17. Fill with solution.	17a. Do not add more water or standard.	
	18. Add the reagent.		
	19. Obtain a value.	19a. Steps 5 through 9.	
	20. Repeat steps 16-19 for the remaining standards.	20a. By comparing standards of known concentrations to the sealed/permanent visual standards and plotting a comparison on graph paper, a correction factor can be derived and applied to all future results obtained on the now calibrated apparatus. 20b. This calibration should be carried out at least every 6 months and checked by running one or two concentrations whenever new reagent (powder or pill) is purchased.	
C. Interference Determination	1. Fill the reference tube with the clear water to be tested.	1a. This procedure must be carried out until it is determined that the interference is not present, then this section may be omitted.	
	2. Rinse the sample tube with the water to be tested.		
	3. Discard this rinse.		
	4. Fill the cell to the mark with sample.	4a. Do not overfill; the reagents are based on this volume.	
	5. Add one small crystal of potassium iodide.		
	6. Add one drop of sodium arsenite solution.	6a. Reagent 5.	
	7. Stopper the tube and mix by inverting several times.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Interference Determination (Continued)	8. Unstopper and add the manufacturer's reagent.	8a. This is the DPD reagent and buffer.	
	9. Stopper and mix by inversion.		
	10. Place cell in comparator.		
	11. Hold comparator up to the light.		
	12. Match any red color in the sample with the standard.		
	13. Record the value.		
	14. Subtract this value from the value obtained in section D.	14a. Step D.10.	
D. Sample Analysis	1. Fill the reference tube with the clear water to be tested.	1a. If a reference tube is used in the kit.	
	2. Rinse the sample tube with the water to be tested.		
	3. Discard this rinse.		
	4. Fill with water to be tested.	4a. Fill to the mark on the tube. Do not over fill; the reagents are based on this volume.	
	5. Add the reagent.		
	6. Stopper and mix by inversion.		

WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Sample Analysis (Continued)	7. Insert into the comparator. 8. Hold the comparator up to the light. 9. Match the color developed in the sample with the standard colors. 10. Check the calibration graph.	10a. If no deviation has been noted, the concentration can be determined directly from the comparator.	
E. Calculations	1. Average the values obtained for each standard. 2. Record averages in Column 5. 3. Plot the known value (Column 3) vs. the average in Column 5.	1a. Use the formula at the top of Column 5 in Table A.b.1c. 3a. Use an arithmetic paper. 3b. See example paper attached.	


Avg. Comparator Values
(Col. 5) mg Cl⁻/1
1.0
0.8
0.6
0.4
0.2
0
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
KNOWN VALUES (COL. 3) mg Cl⁻/1

E13.B-13

WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

DPD KITS

The DPD N.N-diethyl-p-phenylene diamine method was chosen because it is a relatable test and there is a test kit for it. It is specifically mentioned in the comments to the Interim Primary Drinking Water Regulations (p. 59580). The kit can be used for many of the forms of chlorine. However, the only form which must be measured and reported is the free chlorine. The kit requires the addition of a solid reagent to the sample, mixing and comparison of the reading with standard colors, the color generated by the free chlorine and the DPD reagent is formed immediately.

Any kit using the DPD reagent is acceptable for the measurement. Both the Hach Chemical Company and the LaMotte Chemical Company make kits that can be used.

The permanent color standards provided with the kit should be calibrated initially and thereafter periodically to assure correct readings are obtained.

These kits are not to be used to monitor chlorine under the permit system for the National Pollutant Discharge Elimination System (NPDES). This kit is approved only for analysis of chlorine in drinking waters under the National Interim Primary Drinking Water Regulations.

WATER MONITORING PROCEDURE: Measurement of Free Chlorine Utilizing the DPD Kit

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

Training Guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.1.1a	The only interfering substance likely to be encountered in water is oxidized manganese. Consequently an operator should determine if this interfering substance is present or not. If not, the procedure may be omitted. If it is present, the procedure must be carried out prior to each determination.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF TURBIDITY

as applied in

WATER AND WASTEWATER FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.TURB.lab.WMP.1.11.77

WATER MONITORING PROCEDURE: Determination of Turbidity

1. Analysis Objectives:

   The user of the attached material will learn how to use a nephelometric type turbidimeter to measure the turbidity of a sample.

2. Brief Description of Analysis:

   The method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity.

   A person using this outline should have the basic skills used in a laboratory such as:

   a. Preparation of standards
   b. Use of volumetric glassware

3. Applicability of this Procedure:

   a. Range - From 0 to 40 nephelometric turbidity units or with appropriate dilutions higher than 40 units.
   b. Pretreatment of Samples - The sample should be run as soon as possible. Preservation is not recommended.
   c. Treatment of Interferences - (1) Floating debris should be removed before analysis, (2) air bubbles should be allowed to dissipate before reading, (3) with finished waters no other interferences are noted.

Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, Office of Technology Transfer, Cincinnati, Ohio 45268. Page 295.

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WATER MONITORING PROCEDURE: Determination of Turbidity

Equipment or Supply Requirements

A. Capital Equipment:

1. Turbidimeter, Nephelometric - see list of acceptable instruments meeting the following criteria:
    a. Light source - Tungsten lamp operated at not less than 85% of rated voltage or more than rated voltage.
    b. Distance traveled by incident light and scattered light within the sample tube: total not to exceed 10 cm.
    c. Angle of light acceptance of the detector: centered at 90° to the incident light path and not to exceed ± 30° from 90°.
    d. Maximum turbidity to be measured: 40 units.

Acceptable Instruments*

- Hach Model 2100 Model 2100 A	Hach Chemical Co., 713 S. Duff Ave., PO Box 907, Ames, IA 50010
- HF Instruments Model DRT- 15 -100 -150 -200	HF Instruments, Itd.[+], 105 Healey Rd., Bolton, Ontario, Canada
- Turner Model 40-002 (for drinking water) 40-005 (for waste waters)	Turner Designs, 2247A Old Middlefield Way, Mountain View, CA 94043

- Bausch & Lomb - An attachment for their "Spectronic mini 20" Spectrophotometer. This can be obtained from any company that sells Bausch and Lomb Spectrophotometers.

2. Trip balance (or platform) or analytical balance: with 0.01 gram sensitivity
3. Distillation equipment - all glass still or ion exchange cartridges
4. Standard Turbidity Suspensions (optional) - if none supplied with the instrument

B. Reusable Supplies:

1. One brush, bottle
2. One flask, side arm filtering, 500 ml size
3. Six flasks, volumetric, 100 ml size with stoppers
4. One funnel, membrane filter funnel and holder
5. Two pipets, volumetric, 5 ml size
   Three pipets, volumetric, 10 ml size
6. Pipet bulb
7. Wash bottle, squeeze type, 500 ml

[+] Also sold by: Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA 15219

*This list is not meant to be complete. It covers those known by the author at the time of writing this material.

WATER MONITORING PROCEDURE: Determination of Turbidity

C. Consumable Supplies:

1. Distilled water
2. Detergent
3. Membrane filters, 0.45 micron pore size
4. Tissues
5. Weighing boats, plastic disposable, about 12
6. Reagents

Hexamethylenetetramine, reagent grade - can be purchased from:

J. T. Baker Chemical Co.	Cat. No. N145
Fisher Scientific	Cat. No. H289
MC/B or Sargent-Welch	Cat. No. HX-0280
A. H. Thomas Co.	Cat. No. C389

Hydrazine Sulfate, Reagent grade - can be purchased from:

J. T. Baker	Cat. No. 2177
Fisher Scientific	Cat. No. H-320
MC/B or Sargent-Welch	Cat. No. HX-0575
A. H. Thomas Co.	Cat. No. C393

WATER MONITORING PROCEDURE: Determination of Turbidity

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Glassware			
1. Volumetric	1. Wash with brush and detergent.	1a. The glassware used for the formazine preparation may have some polymer adhering to the glass. Consequently, the brush should be used to clean this off.	
	2. Rinse with tap water.		
	3. Rinse with turbidity free water.		
2. Cells	1. Rinse with tap water.	1a. Use care in handling the cells at all times Do not touch them where the light strikes them. 1b. Consult the instrument manual to determine the area where the light strikes the cells. In Hach instruments, it is the bottom of the cell; in the DRT and Turner, it is the side of the cell.	
	2. Rinse with turbidity free water.	2a. See section C.1.	
	3. Handle and dry with soft tissue.		
B. Sample Pre-treatment	1. Measure turbidity as soon as possible after sampling.	1a. Preservation of samples is not recommended. 1b. Within one hour.	VII.B.1 (p. 21)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation			
1. Turbidity Free Water	1. Read the turbidity of the distilled water.	1a. Use directions under "Sample Analysis" Section.	
	2. Filter a quantity of distilled water.	2a. Filter using a vacuum membrane filter apparatus like that used in the membrane filter technique for bacteriological analysis. 2b. The membrane filter should have a pore size of 0.45 micrometer.	
	3. Read the turbidity of the filtered water.		
	4. If the filtered distilled water shows a lower turbidity value, treat all water used in this procedure by filtration.	4a. If the values are the same, use the distilled water without filtration. 4b. Check the distilled water periodically by filtration to assure absence of turbidity.	
2. Hydrazine Sulfate Solution $(NH_2)_2 \cdot H_2SO_4$	1. Weigh out 1.00 grams of hydrazine sulfate.	1a. A trip balance can be used. 1b. Use a plastic weighing boat.	
	2. Transfer the hydrazine sulfate to a 100 ml volumetric flask.	2a. Use a plastic wash bottle and rinse the weighing boat with distilled water. 2b. Add the washings to the volumetric flask. 2c. Rinse three times with about 15 ml's of water.	
	3. Swirl the flask until the hydrazine sulfate has dissolved.		
	4. Dilute with water to the 100 ml mark.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Hexamethylenetetramine Solution $(CH_2)_6N_4$	1. Weigh out 10.00 grams of hexamethylenetetramine.	1a. A trip balance can be used. 1b. Use a plastic weighing boat. 1c. Also called Methenamine.	
	2. Transfer the hexamethylenetetramine to a 100 ml volumetric flask.	2a. Use a plastic distilled water wash bottle and rinse the weighing boat three times. 2b. Add the washings to the volumetric flask. 2c. Each washing should be about 15 ml's.	
	3. Dilute to the mark with distilled water.		
4. Stock Turbidity Suspension (400 units)	1. Pipet 5.0 ml's of the hydrazine sulfate solution into a 100 ml volumetric flask.	1a. Use a 5 ml volumetric pipet. 1b. Always pipet with a pipet bulb. These chemicals are toxic.	
	2. Pipet 5.0 ml's of the hexamethylenetetramine solution into the same 100 ml volumetric flask.	2a. Use a second 5 ml volumetric pipet.	
	3. Mix by swirling the flask.		
	4. Stopper.		
	5. Allow to stand 24 hours.	5a. The temperature should be between 22°C (72°F) and 28°C (82°F). 5b. The formazine polymer forms during this time.	
	6. Remove the stopper.		
	7. After standing, dilute to the 100 ml mark.	7a. With distilled water.	
	8. Stopper.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)	9. Mix well.	9a. Invert the flask several times while holding the stopper in. 9b. This suspension has 400 turbidity units. 9c. This suspension can be kept (when stoppered) for one month.	
5. Standard Turbidity Suspension (40 units)	1. Mix the stock suspension well.	1a. This material will settle out rapidly.	
	2. Pipet 10 ml of the stock turbidity suspension into a 100 ml volumetric flask.	2a. Use a 10 ml volumetric pipet. 2b. Pipet with a pipet bulb.	
	3. Dilute to the 100 ml mark.	3a. With distilled water.	
	4. Stopper and mix well.	4a. This suspension is defined as 40 turbidity units. 4b. This suspension can be kept (when stoppered) for one week. 4c. Never pour the suspension back into the flask after use.	
6. Standard Turbidity Suspension (4 units)	1. Mix the 40 unit standard thoroughly.	1a. By inverting the stoppered flask.	
	2. Pipet 10 ml of the 40 unit standard into a 100 ml volumetric flask.	2a. Use a 10 ml volumetric pipet. 2b. Use a pipet bulb.	
	3. Dilute to the mark.	3a. With distilled water.	
	4. Stopper and mix.	4a. This suspension contains 4 turbidity units. 4b. This suspension can be kept (when stoppered) for one week. 4c. Never pour the suspension back into the flask after use.	

WATER MONITORING PROCEDURE: Determination of Turbidity

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Reagent Preparation (Continued)	5. Label the volumetric flask.		
7. Standard Turbidity Suspension (0.4 units)	1. Mix the 4 unit standard thoroughly.	1a. By inverting the stoppered flask.	
	2. Pipet 10 ml of the 4 unit standard into a 100 ml volumetric flask.	2a. Use a 10 ml volumetric pipet. 2b. Use a pipet bulb.	
	3. Dilute to the mark.	3a. With distilled water.	
	4. Stopper and mix.	4a. This suspension contains 0.4 turbidity units. 4b. This suspension can be kept (when stoppered) for one week. 4c. Never pour the suspension back into the flask after use.	
	5. Label the volumetric flask.		
D. Instrument Calibration	1. Check the meter needle to see that it is on the zero mark.	1a. This is done before the instrument is turned on.	V.D.1 (p. 20)
	2. If it is not, zero it by turning the small screw located on the meter frame.		
	3. Turn on the power switch.	3a. This will sometimes be a separate switch or the instrument is turned on by moving the range selector switch to one of the ranges.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Calibration (Continued)	4. On battery powered instruments, check the batteries.	4a. All battery powered instruments have a battery check position. 4b. See the manufacturer's manual.	
	5. Allow sufficient warm-up time.	5a. This time will vary with the type instrument. Follow the manufacturer's manual. 5b. Line operated instruments should be left on if they are to be used regularly.	
	6. Set the range selector on the 0-1 scale.		
	7. Shake the 0.4 unit standard suspension.	7a. Leave the stopper in place and invert gently several times.	
	8. Wait until large air bubbles disappear.	8a. Do not wait too long or suspension will settle, usually only several seconds.	
	9. Pour into cell.	9a. With the Hach instruments the volume must be 25 ml ± 1 ml. On other instruments the volume is not as critical so long as the cell is filled to about 3/4 of its total volume. 9b. Take care when handling the cell. Do not touch the bottoms of the Hach cells or the lower half of other type cells. Handle all cells by holding the top edges.	
	10. Wipe the cell sides and bottom with a soft tissue.	10a. Take care that it is not scratched.	V.D.10 (p. 20)
	11. Insert the cell into cell compartment.	11a. Handle near the top section only.	
	12. Cover cell compartment.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Calibration (Continued)	13. Allow instrument to stabilize.	13a. Usually in less than one minute. 13b. Stabilization is attained when the needle no longer drifts.	
	14. Turn reference adjust knob until the 0.4 mark is reached.	14a. The meter faces vary in how they are marked off. The user will have to determine which mark represents 0.4 units.	
	15. Turn the range adjust switch to the 0-100 scale.	15a. This step will keep the meter needle from bouncing off the ends of the meter scale. 15b. May be termed the x10 scale.	
	16. Uncover cell compartment.		
	17. Remove standard suspension.	17a. Handle near the top of the container. 17b. Retain this standard for future use.	
	18. Wipe off sides and bottom of the manufacturer's standard with a soft tissue.		
	19. Insert the manufacturer's reference suspension.	19a. If more than one, use the suspension that is nearest to 0.4 units. 19b. Handle near the top section only.	V.D.19a (p. 20)
	20. Cover the cell compartment.		
	21. Turn range adjust switch to 0-1 scale.		
	22. Allow the instrument to stabilize.		
	23. Read the turbidity.	23a. If a discrepancy with the expected reading exists, take note of this reading and proceed.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Calibration (Continued)	24. Turn the range switch to the 0-100 scale.		
	25. Uncover cell compartment.		
	26. Remove manufacturer's standard.		
	27. Shake formazine standard.	27a. The standard used in step 11.	
	28. Wipe with tissue.		
	29. Insert into cell compartment.		
	30. Cover compartment.		
	31. Allow instrument to stabilize.		
	32. Turn range switch to 0-1 scale.		
	33. Read the turbidity.	33a. If it still reads 0.4 units, there is a discrepancy between the formazine and the manufacturer's standard. Note should be taken of how much and whenever the manufacturer's standard is used to calibrate the instrument, this discrepancy should be added or subtracted from its value. 33b. If it no longer reads 0.4, use the reference adjust knob and recalibrate by turning the reference adjust knob until the 0.4 mark is reached. Then repeat these steps beginning at step 15. 33c. Rinse the cell with the next material to be read. When finished, rinse with turbidity free water. Store as manufacturer suggests.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Calibration (Continued)	34. The formazine standards should be used at least once/week to monitor manufacturer's standard for change.		
	35. For future calibration of the 0-1 scale, the manufacturer's standard can be used.	35a. Taking care to allow for any discrepancy noted in step 33. 35b. All ranges on the instrument must be calibrated in this same manner.	
E. Sample Analysis	1. Turn on the instrument.		
	2. Allow warm up time.		
	3. Check batteries.	3a. If the instrument is battery operated.	
	4. Fill a cell with the sample.	4a. Fill about three fourths of the capacity.	
	5. Wipe side and bottom of cell.		
	6. Allow bubbles to disperse.	6a. A light tapping with a finger will speed up this procedure.	
	7. Set the range selector on 0-100 scale.		
	8. Insert cell into cell compartment.		
	9. Cover the cell compartment		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Sample Analysis (Continued)	10. Select the range that will keep the sample's reading on scale and read the turbidity.		
	11. Note which scale is used.	11a. If the value is greater than 40 units, the sample must be diluted before it can be read. 11b. All samples below 40 units should be read on the scale that gives the greatest movement of the meter needle and remains on scale.	
	12. Set range selector on 0-100 range.		
	13. Uncover cell compartment.		
	14. Remove sample.		
	15. Select a manufacturer's standard.	15a. Depending on the manufacturer, the standard could be a simple standard or a set of standards. Choose the standard that will be in the same range as the sample.	
	16. Wipe off side and bottom.		
	17. Insert into cell compartment.		
	18. Cover cell.		
	19. Allow instrument to stabilize.		
	20. Select range used in step 10.		

WATER MONITORING PROCEDURE: Determination of Turbidity

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Sample Analysis (Continued)	21. Adjust reference adjust knob until standard's value is obtained.	21a. Make any change of standard concentration found necessary in step D.33.	
	22. Turn range selector to 0-100 scale.		
	23. Uncover cell compartment.		
	24. Remove standard.		
	25. Insert the cell containing the sample.	25a. Care should be taken not to touch the cell except at the top. If it has been touched anywhere else, wipe the cell off.	
	26. Cover the cell compartment.		
	27. Set scale on range used in step 10 and 20.		
	28. Allow instrument to stabilize.		
	29. Read turbidity.		
	30. Repeat steps 15 through 20.	30a. If the instrument has drifted, from the value set in step 21, recalibrate by adjusting the reference adjust knob and reread the samples turbidity, beginning at step 21.	
	31. Turn range selector to 0-100 scale.		
	32. Uncover cell compartment.		
	33. Remove cell.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Sample Analysis (Continued)	34. Insert next sample or if no more samples are to be read, continue on.	34a. If more samples are to be analyzed, repeat steps 4-10, checking for instrument drift by checking standardization occasionally.	
	35. Cover compartment.		
	36. Turn off instrument.	36a. If line operated, leave power on if instrument is to be used within a reasonable time.	
	37. Wash out cells with turbidity free water.		
	38. Dry cells with soft tissue.		
	39. Store cells as manufacturer recommends.		
F. Calculations	1. Calculate the turbidity of the sample.	1a. Calculation is necessary only where the sample was above 40 units and had to be diluted. 1b. If no dilution was performed, then the turbidity of the sample is read directly from the face of the meter.	IX.F.2c (p. 22)

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>F. Calculations (Continued)</td><td>2. Multiply scale reading times the dilution factor.</td><td>2a. That is $\frac{A \times (B+C)}{C}$
Where B = volume of turbidity free water used to dilute the sample (ml's)
C = the volume of sample used (ml's)
A = the turbidity units of the dilution read on the meter scale
2b. Example:
If 2 ml's of sample were diluted to 10 ml's with turbidity free water and the dilution had a scale reading of 30 units, then
A = 30
B = 8
C = 2
$\frac{10}{2}$ x 30 = 150 turbidity units (TU's)</td><td></td></tr>
<tr><td></td><td>3. Report the results.</td><td>3a. Report as follows:
<table>
<tr><th>TURBIDITY RANGE NTU or TU</th><th>RECORD TO THE NEAREST NTU or TU</th></tr>
<tr><td>0-1.0</td><td>0.05</td></tr>
<tr><td>1-10</td><td>0.1</td></tr>
<tr><td>10-40</td><td>1</td></tr>
<tr><td>40-100</td><td>5</td></tr>
<tr><td>100-400</td><td>10</td></tr>
<tr><td>400-1000</td><td>50</td></tr>
<tr><td>>1000</td><td>100</td></tr>
</table></td><td></td></tr>
</table>

WATER MONITORING PROCEDURE: Determination of Turbidity

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX*	Records and Reports

Training Guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.1	Calibration of any nephelometric turbidimeter should be done using the standard turbidity suspensions prepared as described in the Reagent Preparation Section. Many manufacturers provide secondary standards with purchase of these instruments. It is well to check this by comparing it to a dilution of the standard turbidity suspension. Since the maximum contaminant level is usually one turbidity unit, dilutions of the standard turbidity suspension should be made and calibration of the instrument carried out on the scale near this value. However, each scale of the turbidimeter and each secondary standard should be calibrated against the standard suspension (formazine).	
D.10	The sample cells to be used with the instruments must be of clear colorless glass. They should be kept scrupulously clean both inside and out, and discarded when they become scratched or etched. Some manufacterers claim scratched cells are not a problem, however, this should be verified. They must not be handled at all where the light strikes them, and should be long enough so that they may be handled by the top.	
D.19a	This procedure will calibrate the 0-1 scale and those standards provided by the manufacturer that can be read on that scale. If other scales are to be used, insert a standard suspension (formazine) that was made up in the Reagent Preparation Section that can be read on the scale of interest and set the reference adjust knob to the value.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.1	Turbidity is one of the parameters classed as a health limit in the Interim Primary Drinking Water Regulations. Public water systems are required to measure for turbidity daily. The comments to the Interim Primary Regulations (p. 59581) indicate that turbidity measurements were intended to be carried out by the operators of the public water system. This intent was due to the fact that preservation of the sample was not possible. The location at which the sample is to be taken is listed in the Interim Primary Regulations as the point of entry to the distribution system.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.2c	The faces of the meters are labeled as NTU, TU and FTU. These are: NTU - Nephelometric Turbidity Units TU - Turbidity Units FTU - Formazine Turbidity Units These units are considered as being the same. Express all values obtained as TU's for drinking waters and as NTU's for wastewaters.	

A PROTOTYPE FOR DEVELOPMENT OF
ROUTINE ANALYTICAL PROCEDURES
for the
DETERMINATION OF CHLORINATED HYDROCARBON PESTICIDES

as applied in

POTABLE WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U. S. Environmental Protection Agency

CH.PES.lab.WMP.1.11.77

WATER MONITORING PROCEDURE: Determination of Chlorinated Hydrocarbon Pesticides

Operational Procedures

A. Glassware

B. Reagent Preparation

C. Instrument Set-up

    1. Gas Connection
    2. Detector Installation
    3. Leak Check
    4. Column Conditioning

D. Instrument Calibration

E. Standardization of the Sodium Hydroxide

F. Florisil Preparation

    1. Lauric Acid Value Determination
    2. Testing for Proper Elution Pattern

G. Sample Extraction

    1. Pretreatment
    2. Extraction
    3. Concentration

H. Sample Clean-up

I. Sample Analysis

J. Calculations

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WATER MONITORING PROCEDURE: Determination of Chlorinated Hydrocarbon Pesticides

1. Objective:

To determine the concentration of the chlorinated hydrocarbons, as listed in the Interim Primary Drinking Water Regulations, in water samples.

2. Description of the Analysis:

A measured water sample is extracted with an organic solvent. This chlorinated hydrocarbon containing solvent is then concentrated to a volume of 1 ml. A small volume (10 μl) is injected into a gas chromatograph and the amount of chlorinated hydrocarbon present is quantitated. Should there be interferences present, a method is given to separate the chlorinated hydrocarbon from the interferences. The equations for calculating the μl/liter concentration of chlorinated hydrocarbon are given.

This method is recommended for use only be experienced pesticide analysts or under the close supervision of such qualified persons.

The person attempting to use this outline should have a basic knowledge of gas chromatography. Among these skills should be

a. proper injection technique

b. proper quantitation technique of the peaks

c. knowledge of retention times and relative retention times

d. basic knowledge of the theory and operation of a gas chromatograph

e. basic chemical skills, such as pipetting, solution preparation, etc.

3. Applicability of this Procedure:

a. Range of Concentration

Many of the chlorinated hydrocarbons can be detected at .001 mg/liter quantities. Of those listed in the Interim Primary Drinking Water Regulations, Lindane, Endrin and Methoxychlor can be determined at this level. Using the concentration inherent in the procedure this level can be lowered. Toxaphene, also listed in the Interim Primary Regulations, can be determined but is somewhat more difficult.

b. Pretreatment of Samples

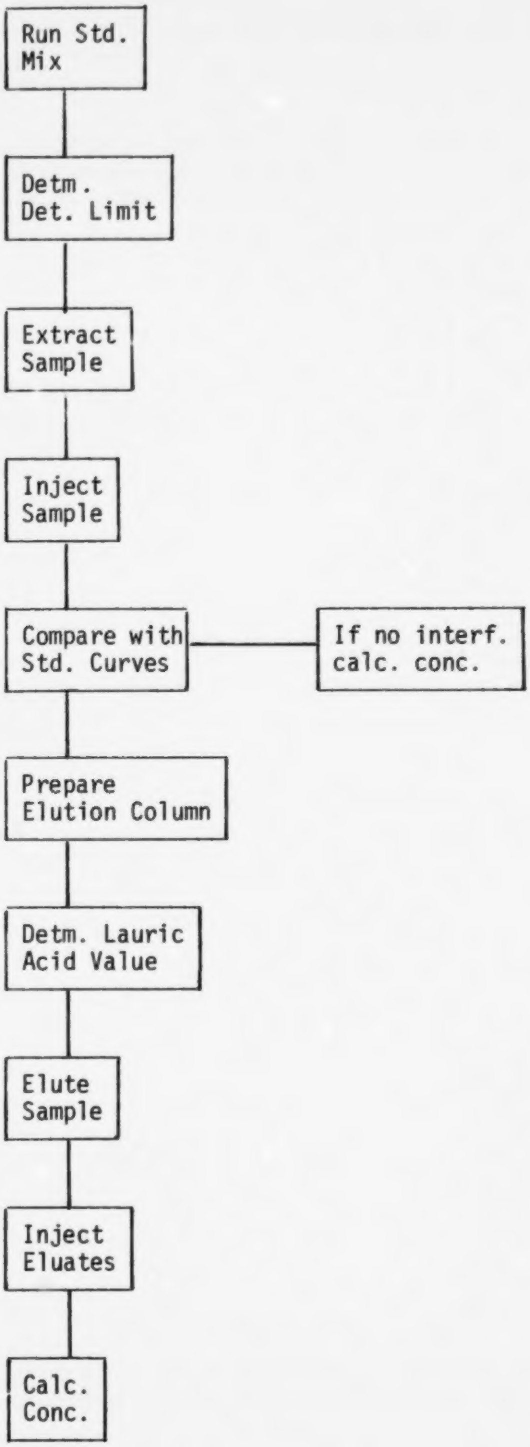
Upon collection of the sample temperature should be lowered to 4° C with ice and maintained at this temperature until analyzed. The maximum holding time is 14 days.

3. Applicability of this Procedure (Continued)

   c. Treatment of Interferences

      This outline includes a clean-up procedure involving separation on a Florisil Column. Inclusion of method blanks during all runs will indicate interferences due to impure solvents and reagents.

Source of Procedure: "Method for Organochlorine Pesticides in Industrial Effluents, EMSL, National Environmental Research Center, Cincinnati, Ohio 45268."


Run Std. Mix
Detm. Det. Limit
Extract Sample
Inject Sample
Compare with Std. Curves
If no interf. calc. conc.
Prepare Elution Column
Detm. Lauric Acid Value
Elute Sample
Inject Eluates
Calc. Conc.

WATER MONITORING PROCEDURE: Determination of Chlorinated Hydrocarbon Pesticides

Equipment and Supply Requirements

A. Capital Equipment:

1. Gas Chromatograph equipped with
    a. Glass lined injection port
    b. Electron capture detector - tritium or nickel 63
    c. Recorder - potentiometric strip chart (10 in - 25 cm) compatible with the detector
2. Gas Chromatographic Column (best purchased from gas chromatographic supply house)
    a. Tubing - Pyrex (180 cm long (6 ft.) x 4mm ID)
    b. Glass Wool - Silanized
    c. Solid Support - Gas Chrom Q (100 - 120 mesh)
    d. Liquid Phase - Expressed as weight percent coated on solid support
        1) OV-1, 3%
        2) OV-210, 5%
        3) OV-17, 1.5% plus QF-1, 1.95%
        4) QF-1, 6% plus SE-30, 4%
3. Hot Water Bath - Capable of keeping temperature at 50° - 100° C
4. Source of high quality distilled water
5. Rotometers - If the instrument is not equipped with meters to monitor the flows of gases, these should be purchased as options
6. Analytical Balance - With a 0.1 milligram sensitivity
7. Trip or Platform Balance - With a 0.1 or 0.01 gram sensitivity
8. Oven - Capable of maintaining 130° C
9. Stop Watch - Capable of measuring at least 1/2 hour, the 60 second cycle divided to 1/5 second
10. Cylinder of Argon-methane (95 + 5%) for use with pulsed mode detector OR Nitrogen - Purified grade, moisture and oxygen free, for use with a DC mode detector
11. Pressure Regulator - Two stage with a CGA 580 fitting for Nitrogen or a CGA 350 fitting for Argon-methane
12. Filter - For carrier gas - molecular sieve type
13. Micro Syringes - 5, 10, 50 µl sizes
14. pH Meter - With pH electrode

WATER MONITORING PROCEDURE: Determination of Chlorinated Hydrocarbon Pesticides

Equipment and Supply Requirements (Continued)

15. Magnetic Stirrer with Teflon coated bar

16. Oven (optional) - Forced air capable of heating to 400° C

17. Trap - for oxygen

B. Reusable Supplies:

1. Beaker, 150 ml - One for each sample - duplicate - blank
2. Beaker, 500 ml - One for each sample - duplicate - blank
3. Buret, 10 ml graduations - One
4. Buret, 25 ml graduations - One
5. Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on the bottom and Teflon stopcock and a 250 ml reservoir bulb at the top of the column with a flared out funnel shape at the top of the bulb, (special order Kontes Glass - K-420540-9011) - One for each sample - duplicate - blank
6. Chromatographic Column - Pyrex (about 400 mm long x 20 mm ID) with coarse fritted plate on bottom - One for each sample - duplicate - blank
7. Cylinders, graduated
   10 ml - One
   50 ml - Three
   100 ml - Two
   250 ml - Two
   1000 ml - One
8. Dropper Bottle, with dropper, 75 ml - One
9. Flasks, Erlenmeyer
   25 ml, glass stoppered - One
   125 ml, glass stoppered - Two
   250 ml, glass stoppered - One
   500 ml, glass stoppered - One
   1000 ml, glass stoppered - One for each sample - duplicate - blank
10. Flasks, volumetric
    10 ml - Five
    100 ml - Three
    500 ml - Three
11. Funnels, Separatory, with Teflon stopcock, 200 ml - One for each sample - duplicate - blank
12. Glassware, Kuderna-Danish (K-D), order from Kontes Glass Company
    a. Concentrator Tube, 10 ml calibrated, Ꞩ joint 12/22 female, #K570050, Size 1025
    b. Snyder Column, three ball, 150 mm long, #K503000, Size 121
    c. Snyder Column, one ball, 150 mm long, #K569001, Size 1/19
    d. Flask, 500 ml volume, #K570001
    e. Stoppers for flask, Ꞩ Size 19/22, #K850500
    One for each sample - duplicate - blank
13. Pipets, graduated
    1 ml - Two
    10 ml - One

Equipment and Supply Requirements (Continued)

14. Pipets, volumetric
    1 ml - One
    5 ml - One
    10 ml - Two
    20 ml - One
15. Reagent Bottles, glass, glass stoppered
    100 ml - One
    500 ml - Four
    1000 ml - One
16. Ring stand with ring and clamp and base - One for each sample - duplicate - blank
17. Ruler, divided in millimeters, about 30 cm long
18. Stirring Rod, glass, 12 in. long
19. Safety Glasses
20. Bottle, glass, wide-mouth, with glass stopper, 500 ml - One
21 Desiccator

C. Consumable Supplies:

1. Alcohol, ethyl, U.S.P. or absolute, neutralized to phenolphthalein (see Reagent Preparation Section)
2. Ethyl ether, Nanograde, pesticide quality
3. Florisil, PR Grade (60-100 mesh), purchase activated at 1250° F. Store in the dark in glass containers
4. Hexane, Nanograde, distilled in glass
5. Lauric Acid, purified, CP
6. Methylene Chloride, Nanograde, distilled in glass
7. Pesticide Standards, reference grade
8. Petroleum Ether, (boiling range 30-60° C) Nanograde (98+ % pure)
9. Phenolphthalein Indicator
10. Soap Solution, any liquid soap mixed 1:1 with water
11. Sodium Hydroxide, ACS
12. Sodium Sulfate, ACS, Granular, anhydrous
13. Sulfuric Acid, ACS
14. Distilled Water
15. Weighing Boats, plastic disposable
16. Chart Paper, for the recorder
17. Notebook, bound
18. Paper, graph

WATER MONITORING PROCEDURE: Determination of Chlorinated Hydrocarbon Pesticides

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Glassware Preparation	1. Clean all Glassware.		
	2. Wash with soap and water.		
	3. Rinse with tap water.	3a. At least 10 times.	
	4. Rinse with distilled water.	4a. At least 10 times.	
	5. Muffle at 400° C for 15 to 30 min.	5a. Volumetric glassware should not be muffled. 5b. Plastic ware and cap liners for sample containers should not be muffled. 5c. The glassware may be rinsed with redistilled acetone followed by a rinse with pesticide quality hexane in place of the muffling.	
	6. Cool to room temperature.		
	7. Store until used.	7a. Store inverted or cover mouth with aluminum foil. 7b. Sample containers should be stored capped.	
B. Reagent Preparation 1. Ethyl Ether (6%) in Petroleum Ether	1. Use caution with this solvent.	1a. Prepare and use in a well ventilated area. 1b. Prepare just before use.	
	2. Add about 100 ml of petroleum ether to a 250 ml graduated cylinder.		
	3. Pipet 12 ml of ethyl ether into the graduated cylinder.		
	4. Dilute to 200 ml with petroleum ether.	4a. In the graduated cylinder.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	5. Mix with a long glass stirring rod.	5a. Do not store this solution. Prepare and use immediately.	
2. Ethyl Ether (15%) in Petroleum Ether	1. Use the above directions except in Step 3, pipet 30 ml of ethyl ether.	1a. For water supply monitoring for the pesticides listed in the Interim Primary Regulations only the 6% and 15% should be needed. However, if wastewaters are being monitored a 50% eluate will have to be prepared and used. 1b. Do not store this solution.	
3. Alcohol - Ethyl Neutralized to Phenolphthalein	1. Measure 500 ml of alcohol into a 1 liter Erlenmeyer flask.		
	2. Add 3 drops of phenolphthalein indicator.		
	3. Titrate with 0.05 N Sodium Hydroxide until a pale red color is obtained.		
	4. Store in glass stoppered reagent bottle.		
4. Phenolphthalein Indicator	1. Weigh out 1.0 g of the phenolphthalein indicator.	1a. Use a trip balance.	
	2. Transfer to a 100 ml volumetric flask.		
	3. Dissolve in about 50 ml ethyl alcohol.		
	4. Dilute to the mark with distilled water.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued) 5. Lauric Acid Solution (2% W:V)	1. Weigh out 10.000 g of lauric acid.	1a. Use an analytical balance.	
	2. Add about 250 ml hexane to a 500 ml volumetric flask.	2a. Just add hexane to the volumetric flask until half full.	
	3. Transfer the lauric acid into the 500 ml volumetric flask.		
	4. Wash the weighing container with several small portions of hexane and add to the volumetric flask.		
	5. Dissolve the lauric acid.		
	6. Dilute to the mark with hexane.	6a. 1 ml = 20 mg lauric acid.	
	7. Mix thoroughly.		
	8. Store in a glass stoppered bottle.		
6. Methylene Chloride (15%) in Hexane (V:V)	1. Add about 200 ml hexane to a 500 ml graduated cylinder.		
	2. Measure 75 ml of methylene chloride.	2a. With a graduated cylinder.	
	3. Add the methylene chloride to the hexane.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	4. Dilute to the mark with hexane.		
	5. Mix.		
	6. Store in a glass, glass stoppered bottle.		
7. Sulfuric Acid (H_2SO_4) 1:1 (V:V)	1. Add about 25 ml of distilled water to a 100 ml volumetric flask.		
	2. Measure 50 ml concentrated sulfuric acid in a 100 ml graduated cylinder.	2a. Caution: Use safety glasses. 2b. Do not add water to acid; follow the procedure.	
	3. Add the sulfuric acid to the volumetric flask.	3a. The solution will get hot; cool to room temperature before proceeding.	
	4. Dilute to the mark.	4a. With distilled water.	
	5. Cool.		
	6. Check to assure the volume is still on the mark.	6a. If not, add water to mark.	
	7. Store in a glass stoppered bottle.		
8. Sodium Hydroxide (NaOH) 10 N	1. Weigh out 40 g of Sodium Hydroxide.	1a. Use a trip balance. 1b. Weigh out in a 250 ml Erlenmeyer flask.	
	2. Measure 100 ml of water.	2a. In a graduated cylinder.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	3. Add the water to the Erlenmeyer flask.	3a. The solution will get hot; cool under cold water. 3b. Use safety glasses.	
	4. Swirl to dissolve.	4a. Do not stopper and invert to mix. 4b. Use a stirring rod or magnetic stirrer and Teflon coated bar.	
	5. Stopper the flask with a rubber stopper and label.	5a. Do not stopper unless cool.	
9. Sodium Hydroxide (NaOH) 1.0 N	1. Weigh out 20 g of sodium hydroxide.	1a. Use a trip balance. 1b. Weigh out in a plastic weighing boat.	
	2. Transfer the NaOH to a 500 ml Erlenmeyer flask.		
	3. Measure 500 ml of water.	3a. Use a 500 ml graduated cylinder.	
	4. Add the water to the Erlenmeyer flask.	4a. The solution will get hot; cool under cold water. 4b. Use safety glasses.	
	5. Swirl to dissolve.	5a. Do not attempt to stopper and mix by inversion, use a stirring rod or a magnetic stirrer and Teflon coated bar.	
	6. Stopper the flask with a rubber stopper and label.	6a. Do not stopper unless cool.	
10. Sodium Hydroxide (NaOH) (0.05 N)	1. Add about 250 ml distilled water to a 500 ml volumetric flask.		
	2. Transfer 25 ml of the 1.0 N NaOH to the volumetric flask.	2a. Use a 25 ml volumetric pipet.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	3. Dilute to the mark.		
	4. Mix thoroughly.		
	5. Store in a glass, rubber stoppered, bottle.		
11. Sodium Sulfate	1. Weigh out 1.0 gram of the sodium sulfate.	1a. Use a trip balance. 1b. Weigh in a 150 ml beaker.	
	2. Add enough methylene chloride - Hexane (Reagent 6) to cover the sodium sulfate.		
	3. Mix.		
	4. Decant reagent.	4a. Take care not to include any sodium sulfate.	
	5. Inject 10 µl of the solvent into the gas chromatograph.	5a. After retention times and optimization have been determined.	
	6. If contaminated proceed as below.	6a. Contamination is shown peaks of 2 times the noise level are obtained.	
	7. Weigh out about 100 g of sodium sulfate in a 500 ml beaker.	7a. Use a trip balance, weigh in the beaker.	
	8. Place beaker and sodium sulfate in an oven.	8a. The oven should be preheated to 400° C.	
	9. Heat the sodium sulfate for 4 hours at 400° C.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	10. Remove from oven and place in a desiccator and allow to cool.	10a. Use tongs to handle the hot beakers. 10b. Be sure the desiccant is activated.	
	11. Transfer to a wide-mouth glass stoppered bottle.		
	12. Store in the bottle in a desiccator.		
12. Pesticide Standards	1. Consult Tables 1 and 2	1a. In order to properly calibrate the instrument both qualitatively (Retention Values) and quantitatively (Detection Limits) single standard and a mixture containing all the pesticides of interest should be made. 1b. The amount weighed out to each compound was kept at 10 mg in order to have a weight which could be accurately weighed yet using the smallest amount of compound possible. 1c. After weighing out 10 mg and preparing 10 ml of the stock solution of each pesticide, proceed toward the right of the table and make two dilutions as directed. 1d. Use Table 2 for preparing two mixtures. Mixture 1 is used to prepare the column. Mixture 2 will be used to determine the standard curves.	I.B.12

DIRECTIONS FOR DILUTION TABLE

In order to properly calibrate the instrument both qualitatively (Retention Values) and quantitatively (Detection Limits) a single standards and a mixture containing all the pesticides of interest should be made.

The amounts weighed out of each compound was kept at 100 mg in order to have a weight which could be accurately weighed yet using the smallest amount of compound possible.

After weighing out and diluting the stock solution of each pesticide, proceed toward the right of the chart. Making dilutions as directed.

Use Table 2 for preparing two mixtures. Mixture 1 is used to prepare the column. Mixture 2 will be used to determine the standard curves.

TABLE 1

Compound	Stock	Dilution 1	Conc. mg/l	Dilution 2	Conc. mg/l
Lindane	$\frac{\text{10 mg}}{\text{10 ml*}}$	$\frac{\text{4 ml stock}}{\text{100 ml**}}$	40	$\frac{\text{1 ml Dil. 1}}{\text{100 ml}}$	0.4
Endrin	$\frac{\text{10 mg}}{\text{100 ml*}}$	$\frac{\text{2 ml stock}}{\text{100 ml**}}$	20	$\frac{\text{1 ml Dil. 1}}{\text{100 ml}}$	0.2
Methoxychlor	$\frac{\text{10 mg}}{\text{10 ml**}}$	$\frac{\text{1 ml stock}}{\text{100 ml**}}$	10	None	None
Aldrin	$\frac{\text{10 mg}}{\text{10 ml*}}$	$\frac{\text{1 ml stock}}{\text{100 ml**}}$	10	$\frac{\text{1 ml Dil. 1}}{\text{100 ml}}$	0.1
Toxaphene	$\frac{\text{10 mg}}{\text{10 ml*}}$	$\frac{\text{5 ml stock}}{\text{100 ml**}}$	50	$\frac{\text{1 ml Dil. 1}}{\text{100 ml}}$	0.5

* Make stock solutions in 2.2.4 Trimethyl pentane (Isooctane).

** Use hexane for all dilutions of the stock.

TABLE 2

Use Hexane for all dilutions

Compound	Mixture 1 Add to the same 5 ml vol. flask Amount ml.	Dilution No.	Conc. mg/l	Mixture 2 Add to the same 100 ml vol. flask Amount ml.	Dilution No.	Conc. mg/l
Lindane	1.0	Stock	200	1	1 (40 mg/l)	0.4
Endrin	1.0	Stock	200	1	1 (20 mg/l)	0.2
Methoxychlor	1.0	Stock	200	1	Stock (1000 mg/l)	10.0
Aldrin	1.0	Stock	200	1	1 (10 mg/l)	0.1
Toxaphene	1.0	Stock	200	1	1 (50 mg/l)	0.5

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Instrument Set-up 1. Gas Connection	1. Remove cylinder cap from cylinder of carrier gas.	1a. The tank should be chained to the wall or lab bench. 1b. Use Argon-methane for pulsed mode detector or Nitrogen for detector operated in a DC mode. Consult the manufacturer's manual.	V.C.1 (p. 45)
	2. Install the pressure regulator.	2a. The regulator should have a CGA 350 fitting for Argon-methane or for Nitrogen. 2b. Fitting should be tight to a CGA 350 fitting to prevent leaks.	
	3. Connect the cylinder to the instrument.	3a. Use teflon tape on all metal threads to prevent leaks. 3b. Polethylene (1/8" diameter) tubing can be used. 3c. If plastic tubing is used, nylon ferrules should be used with the connector fittings.	
2. Detector Installation	1. Install the electron capture detector.	1a. This can be of the tritium or nickel 63 type. 1b. See the manufacturer's manual on procedures for installation.	
3. Carrier Gas Leak Check	1. Open master valve on the cylinder.		
	2. Adjust regulator control.	2a. To about 65 psig and allow to stabilize (about 1 minute).	
	3. Close carrier gas control valve on the instrument.		
	4. Turn off master cylinder valve.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Instrument Set-up (Continued)	5. Observe gage on the cylinder.	5a. Pressure should not drop more than a few psig. 5b. If the pressure does drop use soap solution to locate leak. 5c. Correct leak and check again.	
	6. Use soap solution and check for leaks at the injection port and column connections.		
4. Column Conditioning	1. Install packed column in the oven by connecting only the column inlet.	1a. These columns should be purchased and meet the specifications as listed under the equipment section. 1b. Column conditioning is essential to eliminate column bleed and to provide acceptable analysis. 1c. Do not connect the column to the detector. 1d. If in doubt as to column installation refer to the manufacturer's manual.	
	2. A flow of carrier gas should be started through the detector.	2a. Use the purge gas line or in dual column oven by connecting an unpacked column to the detector. 2b. In some systems it may be necessary to temporarily connect the carrier gas to the air or hydrogen inlet in order to get a flow to the detector. The manufacturer's manual should be consulted.	
	3. Begin a low flow of carrier gas through the column.	3a. Less than 60 ml/min.(N40-50). 3b. Removes oxygen and other trapped gases. 3c. This will be two separate flows. The column should not be connected to the detector.	
	4. Wait 5 minutes.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Instrument Set-up (Continued)	5. Turn on power to the oven.	5a. Consult manufacturer's manual for location and necessary steps.	
	6. Adjust column temperature to near the maximum recommended temperature for the liquid phase being used.	6a. Column — Max. Temp. ° C OV-17 & QF-1 — 250 OV-210 — 275 OV-1 — 350 QF-1 & SE-30 — 250	V.C.6.b (p. 45)
	7. Continue heating for 2 hours.		
	8. Reduce temperature to about 40° C below maximum temperature.	8a. See table above (6a).	
	9. Allow column temperature to equilibrate.	9a. Minimum of 30 minutes. 9b. Caution: Bleed off of the liquid phase will occur if the column temperature is not fully equilibrated.	
	10. Check carrier gas flow.	10a. About 50 ml/min. 10b. If the instrument does not come equipped with rotometers to monitor the flow rate of the carrier gas this should be purchased as an option. 10c. The pressure regulator on the cylinder should be set at 65 psig. 10d. The electron capture detector must be installed.	
	11. Allow to remain at temperature and flow for one hour.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Instrument Set-up (Continued)	12. Increase temperature to about 20° C above operating temperature.	12a. This is operation temperature not maximum temperature. 12b. These temperatures would be 200° C for the OV-17 and QF-1 and the QF-1 & SE-30 OR 200° C for the OV-210 and the OV-1.	
	13. Continue the same flow of carrier gas.		
	14. Allow to equilibrate for 24-48 hours.	14a. Caution: Do not exceed maximum recommended temperatures. See 6a. this section.	
	15. Turn off oven and allow to cool to room temperature.		
	16. Adjust carrier gas flow to method flow rate.	16a. 60 ml/min. for the OV-17 & QF-1 and OF-1 & SE-30. 70 ml/min. for the OV-210 and OV-1.	
	17. Connect column to the detector.	17a. Check connection with soapy solution.	
	18. Turn on oven and adjust to method column temperature.	18a. 200° C for the OV-17 & QF-1 and the QF-1 & SE-30. 180° C for the OV-210 and the OV-1.	
	19. Allow the instrument to equilibrate at least one hours.	19a. Preferably overnight.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Calibration 1. Optimization	1. Check instrument operating conditions after the instrument has been set-up.	1a. The oven temperature should be stabilized. 1b. The flow rate constant. 1c. The column conditioned. 1d. The flow system checked for leaks.	
	2. Inject 5 µl of standard mixture 2.	2a. This standard mixture was prepared from the stock standards (Table 2). 2b. The actual volume of material injected should be kept constant. That is, the same for standards and samples. 2c. The standard mixture 2 can be used to optimize the instrument initially and thereafter monitor its performance.	
	3. Adjust the operating parameters to achieve optimum results.	3a. Best resolution (separation of peaks) and retention times can be achieved by adjusting the column temperature and/or the carrier gas flow rate.	V.D.1.3a (p. 45)
		3b. Compare results with the standard chromatograms attached. 3c. Caution should be taken to allow the instrument to equilibrate after any changes. 3d. Optimum results would include good separation of the peaks, good sensitivity, good reproducibility.	
2. Retention Time Determination	1. Inject 5 µl of dilution 2 (Table 1) of the standards one at-a-time.	1a. The individual standards lindane, endrin, methoxychlor, aldrin, and toxaphene should be used. 1b. Dilutions of the individual standards can be prepared from the stock solutions as in Table I.	
		1c. Injection is a technique which must be learned and practiced in order to make accurate and reproducible injections.	VII.D.4 (p. 46)
		1d. The analyst should consult a text on gas chromotography or a syringe manufacturer's literature.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Calibration (Continued)	2. With a stop watch measure the time elapsed between the first appearance of the solvent peak and the peak of the known standard.	2a. This time is called the retention time. 2b. After the retention times have been obtained using single standards, the mixtures should be used to determine that no changes in retention time occur.	
	3. Repeat steps 1 and 2 six more times.	3a. At least 7 repeat times should be obtained and the mean value obtained for each pesticide. 3b. If in subsequent injections, the retention times vary significantly (± 2%) the system should be checked over.	
3. Detection Limit of the Instrument	1. Inject 5 µl of a single pesticide standard.	1a. Begin with Dilution 2, Table 1 of each. 1b. The standards need not be carried through the extraction and concentration steps to develop a standard curve.	
	2. Continue with each standard by diluting the last standard concentration run in half and injecting 5 µl portions until a detection limit is obtained.	2a. The detection limit is usually considered as a standard whose peak is 2 times the highest peak caused by noise and is run at the most sensitive setting of the instrument.	V.D.3.2 (p. 45)
	3. The peak height in millimeters should be used and plotted against the known concentrations to produce a standard curve.	3a. The curve produced can be used to select the concentration of standard to be injected to calculate the concentration of an unknown. See calculation of results section. 3b. To select the concentration to be used to calculate the unknown, first note the peak height of the unknown. Second refer to this standard curve and find a concentration which gave the same peak height and inject it into the instrument. This should provide a standard very close to the sample peak height to use in the calculations.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTE
E. Standardization of Sodium Hydroxide	1. Weigh out 100 mg of lauric acid.	1a. On an analytical balance.	
	2. Quantitiatively transfer to a 125 ml Erlenmeyer flask.		
	3. Dissolve with 50 ml ethyl alcohol.	3a. Neutralized to phenolphthalein (See B.3).	
	4. Add 3 drops phenolphthalein.		
	5. Titrate with (0.05 N) sodium hydroxide.	5a. See reagent section for preparation. 5b. Until a pink color persists.	
	6. Calculate mg lauric acid/ml 0.05 N NaOH.	6a. $\frac{\text{mg lauric acid}}{\text{ml of 0.05 N NaOH}} = \frac{\text{100 mg lauric acid}}{\text{number of ml needed to titrate (from Step 5)}}$. 6b. See calculation section J.1 for use.	
F. Florisil Preparation 1. Lauric Acid Value Determination	1. Place 2.000 grams of Florisil in a 25 ml glass stoppered Erlenmeyer flask.	1a. Weigh on an analytical balance.	VII.F.1 (p. 46)
	2. Cover the flask loosely with foil.		
	3. Heat overnight at 130° C.		
	4. Stopper and cool to room temperature.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Florisil Preparation (Continued)	5. Remove stopper and add 20.0 ml of lauric acid solution (reagent 5).	5a. Use a 20 ml volumetric pipet.	
	6. Stopper the flask.		
	7. Swirl intermittently for 15 minutes.	7a. A gentle swirling of the flask is sufficient. 7b. This is to assure contact of the florisil with the lauric acid solution.	
	8. Allow the florisil to settle.		
	9. Pipet 10.0 ml of the supernatant into a 125 ml Erlenmeyer flask.	9a. Use a 10 ml volumeteric pipet. 9b. Supernatant is the clear liquid standing above the settled absorbent. 9c. Avoid inclusion of any florisil.	
	10. Add 50 ml ethyl alcohol.	10a. Neutralized to phenolphthalein. 10b. See reagent section for preparation.	
	11. Add 3 drops of phenolphthalein indicator.	11a. The solution should remain colorless.	
	12. Titrate with 0.05 N NaOH to a permanent red color.	12a. Those individuals not familiar with procedures for titration should consult a procedure on the use of a buret.	
	13. Calculate the amount of lauric acid absorbed by the florisil.	13a. The calculation will be found under the calculation section of this procedure. 13b. This lauric acid value must be obtained for each new batch of florisil purchased. Then an equivalent weight of the new batch can be calculated to obtain values similar to the old batch.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Florisil Preparation (Continued)	14. Store the rest of the florisil.	14a. In glass bottle with glass stopper kept in the dark in an oven at 130° C.	
2. Testing for Proper Elution Pattern	1. Weigh out the determined amount of florisil.	1a. Determined from the lauric acid value of the batch of florisil. 1b. Usually about 10 to 15 grams. 1c. See the calculation section (J.2).	VII.F.2 (p. 46)
	2. Pour the florisil into the chromatographic column.	2a. The column should meet the specifications listed in the equipment section.	
	3. Tap the column lightly to settle the florisil and to level its surface.	3a. The column should not be packed so tight as to impede solvent flow.	
	4. Add about one-half inch of sodium sulfate to the top of the florisil in the column.	4a. This should be the anhydrous, granular, ACS grade. 4b. Should be pre-conditioned by heating at 400° C for four hours (See B.11).	
	5. Add 40-50 ml of petroleum ether to the top of the column.	5a. This is used to pre-wet the column. 5b. The column may generate heat as it is wet by the solvent. Let cool to room temperature before proceeding. 5c. This solvent can be collected in any type of container and need not be saved.	
	6. Time and rate of passage of the petroleum ether through the column.	6a. This elution rate should be set at about 5 ml per minute. 6b. Use a 10 ml graduated cylinder and a stopwatch.	
	7. Remove the container used to collect the petroleum ether.	7a. This should be done while a small level of solution is still above the sodium sulfate layer.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Florisil Preparation (Continued)	8. Place a clean 500 ml K-D flask equipped with a 10 ml graduated ampul under the column.	8a. Some portion of the petroleum ether will be collected. 8b. K-D is a Kuderna-Danish.	
	9. Just prior to the exposure of the sulfate layer to the air add 10 ml of mixture 2.	9a. See the reagent preparation section (B.12, Table 2). 9b. The sulfate surface should not be allowed to dry between additions of the mixture and the following eluates.	
	10. Rinse container with 10 ml of petroleum ether.	10a. Add the rinse to the column.	
	11. Add 200 ml of the 6% ethyl ether in petroleum ether solution.	11a. Measure in a graduated cylinder. 11b. If prepared just prior to use by the directions given in the reagent preparation section, only 200 ml will be prepared and can be completely transferred to the column. 11c. Add small portion slowly to bring liquid level to the top of the florisil column then add the rest.	
	12. Collect the 200 ml	12a. Close the column's stopcock before the sulfate layer is exposed to the air.	
	13. Add 200 ml of the 15% ethyl ether-petroleum ether solution.		
	14. Immediately replace the 500 ml K-D flask with another clean one.		

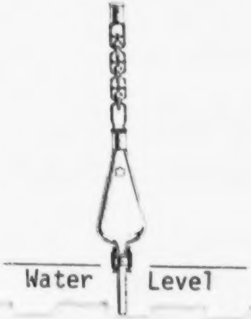
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Florisil Preparation (Continued)	15. Collect the 15% eluate.	15a. There will be a small overlap; in this case a small portion of the 6% will be collected in the 15% flask. Again close the stopcock to prevent exposure of sulfate layer to the air.	
	16. Just prior to the sulfate layer being exposed add 200 ml of ethyl ether.	16a. For wastewater or if other pesticides are to be monitored other than those listed in the Interim Primary Regulations, a 50% ethyl ether in petroleum ether elution would be carried out at this time followed by the straight ethyl ether elution.	
	17. Immediately replace the 500 ml K-D flask with another clean one.		
	18. Collect the ethyl ether elution.		
	19. Insert a Snyder column into each flask.		
	20. Concentrate each elution.	20a. Use the procedure under sample concentration, G.3, 1 through 6.	
	21. Remove the flask from the ampul.	21a. Rinse bottom of flask and lower glass joint into the ampul.	
	22. Rinse the walls of the ampul.	22a. Rinse to a final volume of 10 ml. 22b. Use a 5.0 cc glass syringe.	
	23. Stopper the ampul.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Florisil Preparation (Continued)	24. Inject 5 µl of the fractions.	24a. The pattern will be 6% eluate Aldrin-Lindane-Methoxychlor-Toxaphene, 15% eluate Endrin, Ethyl ether and anything left on the column.	
	25. Compare retention times with standards run in Section D.5.	25a. If agreement is not attained, check the instrument operational parameters (Section D.1).	
G. Sample Extraction and Concentration 1. Pre-Treatment	1. Blend the sample.	1a. This is usually not required for drinking waters.	
	2. Adjust pH to 6.5 - 7.5	2a. Use 50% sulfuric acid or 1.0 N sodium hydroxide. 2b. Use a pH meter to measure the pH. 2c. Usually not necessary for drinking waters.	
2. Extraction	1. Measure out 1 liter of sample.	1a. Use a 1 liter graduated cylinder. 1b. Experience with the sample source will indicate if smaller volumes should be used. If smaller volumes are used they should be diluted to 1 liter volume before extraction. 1c. A 1 liter volume of distilled water should be carried through the entire procedure along with each sample batch to serve as a method blank. 1d. The standard mixture 2 to be used as a check on the curve (D.3.3) must also be carried through this procedure. 1e. Duplicate analysis of the samples are recommended to be carried out.	
		1f. Surface waters sometimes require larger volumes.	VII.G.2.1f (p. 46)
	2. Transfer to a 2 liter separatory funnel.	2a. Use a 2 liter size in order to have room to obtain proper mixing.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Sample Extraction and Concentration (Continued)	3. Add 60 ml of the methylene chloride-hexane mixture (Reagent 6) to the separatory funnel.	3a. Use the 60 ml to rinse the sample container (if empty) and the graduated cylinder.	
	4. Shake vigorously for two minutes.	4a. Pressure may build up in the separatory funnel. Invert, with stopper tightly in place, open stopcock slowly to relieve pressure, do this several times during shaking.	
	5. Return to holder and allow mixtures to separate.	5a. The water layer will be on the bottom and the methylene chloride - hexane on top. 5b. The holder is some type support, such as a ring clamped to a ring stand.	
	6. Prepare the anhydrous sodium sulfate column.	6a. Add 3 to 4 inches of anhydrous sodium sulfate to the chromatographic column (Pyrex, approximately 400 mm long x 20 mm I.D. with a coarse frit on the bottom). 6b. The column should be placed in a support such as a clamp attached to a ring stand. 6c. Position the bottom of the column well into the neck of a 500 ml Kuderna-Danish flask with a 10 ml graduated ampul attached to the stand. 6d. The sodium sulfate should have been heated at 400° C for four hours in an oven (Section B.11).	
	7. Remove the stopper from the separatory funnel.		
	8. Drain the water layer into a one liter Erlenmeyer flask.	8a. Keep the water layer for further extractions.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Sample Extraction and Concentration (Continued)	9. Pour the organic (methylene chloride-hexane) layer into the sodium sulfate column.	9a. Pour from the top of the separatory funnel. 9b. Use a funnel at the top of the column to help transfer sample into column.	
	10. Collect the organic layer after it has passed through the column in a 500 ml Kuderna-Danish (K-D) flask with a 10 ml ampul attached.		
	11. Return the water phase to the separatory funnel.		
	12. Rinse the Erlenmeyer flask with a second 60 ml portion of the methylene chloride-hexane solvent.		
	13. Transfer the second portion of the methylene chloride-hexane solvent to the separatory funnel.		
	14. Put stopper in place.		
	15. Shake vigorously for two minutes.	15a. Release pressure periodically.	
	16. Repeat steps 5 and 7 through 11.	16a. Step 6 does not have to be done again. Use the same column. 16b. Collect the second organic layer (methylene chloride-hexane) in the same K-D flask.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Sample Extraction and Concentration (Continued)	17. Perform the extraction procedure with a third portion of the methylene chloride-hexane solution.	17a. Steps 12 through 17. 17b. All three 60 ml organic extraction portions are combined in the K-D flask.	
	18. Rinse the sodium sulfate column three times with 10 ml volumes of methylene chloride-hexane.	18a. Collect in the K-D. flask.	
3. Concentration	1. Insert a condenser into the K-D flask.	1a. Condenser - Snyder column - three ball.	
	2. Place K-D flask in a holder above a boiling water bath.		
	3. Add small boiling chip.		
	4. Lower the ampul into the water.	4a. The water level should be maintained below the lower joint (where the ampul connects to the flask). The lower rounded surface of the flask should be bathed in steam. 4b. Surrounding the flask with aluminum foil will help. 4c. The evaporation must not go to dryness. The analyst should stay with the flask.	



OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Sample Extraction and Concentration (Continued)	5. Allow the solvent to evaporate.	5a. Evaporation should be adjusted so that the solvent vapors are rushing through the condenser. 5b. Carry out the evaporation in a hood. 5c. There should be no splashing at the top of the column or flooding of the chambers.	
	6. Concentrate to about 1 ml volume.	6a. This will be in the ampul. However, after cooling solvent that has remained in the Snyder column will drain back into the ampul and raise the volume to above 1 ml.	
	7. Remove from the bath.		
	8. Remove the three ball condenser from the flask.		
	9. Rinse the lower joint of the column into the ampul.	9a. Rinse with hexane.	
	10. Insert a micro Snyder column (one ball) into the ampul.		
	11. Return to the water bath.	11a. Caution: When using the micro column, the analyst should constantly watch the samples as they are heated. They must not go to dryness.	
	12. Boil and reduce the volume to about 0.2 to 0.5 ml.	12a. Do not allow to go to dryness. 12b. After cooling the volume will increase by about 0.1 ml by the solvent draining back into the ampul.	
	13. Cool.		
	14. Remove the micro column.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Sample Extraction and Concentration (Continued)	15. Rinse the lower joint of the column into the ampul.	15a. Use a 10 µl syringe. 15b. The rinse should be about 0.1 to 0.2 ml. 15c. Caution: Do not go above 1 ml.	
	16. Dilute to the final volume of 1 ml.	16a. With hexane. 16b. This would include samples, check standards, duplicates and method blanks.	VII.G.3.16 (p. 46)
	17. Stopper to prevent further evaporation.		
	18. Inject 5 µl of this concentration into the gas chromatograph.	18a. This chromatogram will provide the analyst with the information for further need of concentration or clean-up. 18b. Interferences in the form of distinct peaks and/or high background will indicate further clean-up is necessary.	
	19. If interferences are present proceed to the clean-up section.	19a. See next section (Section H).	
	20. If no interferences are present and pesticides are determined, proceed to the calculation section.	20a. If a standard of a concentration at the drinking water MC has been carried through the procedure and gives quantitatable peaks, the analyst can express his value as less than his detection limit if he has obtained no peaks in the sample. 20b. Both the sample and duplicate should produce the same results. 20c. Section J.	I.B.12 (p. 44)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Sample Clean-Up	1. Weigh out the determined amount of florisil.	1a. Determined from the lauric acid value for this batch of florisil. 1b. Usually about 10 to 20 grams. 1c. See calculation section (J.2).	
	2. Pour the florisil into the chromatographic column.	2a. The column should meet the specifications listed in the equipment section.	
	3. Tap the column lightly to settle the florisil and to level its surface.	3a. The column should not be packed so tight as to impede solvent flow.	
	4. Add about one-half inch of sodium sulfate to the top of the florisil in the column.	4a. This should be the anhydrous, granular, ACS grade 4b. Should be pre-conditioned by heating at 400° C for four hours (See B.11).	
	5. Adjust the sample(s) volume to 10 ml.	5a. A column must also be prepared for the method blank, the standard check and each duplicate being run. 5b. Use hexane.	
	6. Add 40-50 ml of petroleum ether to the top of the column.	6a. This is used to pre-wet the column 6b. The column may generate heat as it is wet by the solvent. Allow to cool to room temperature before proceeding. 6c. This solvent can be collected in any type of container and need not be saved.	
	7. Time the rate of passage of the petroleum ether through the column.	7a. This elution rate should be set at about 5 ml per minute. 7b. Use a 10 ml graduated cylinder and a stopwatch.	
	8. Remove the container used to collect the petroleum ether.	8a. This should be done while a small level of solution is still above the sodium sulfate layer.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Sample Clean-Up (Continued)	9. Place a clean 500 ml K-D flask equipped with a 10 ml ampul under the column.	9a. Some portion of the petroleum ether will be collected.	
	10. Just prior to the exposure of the sulfate layer to the air quantitatively transfer the sample extract into the column.	10a. The sulfate should not be allowed to dry between additions of the sample or future eluates. 10b. Wash the ampul with three 5 ml portions of petroleum ether and transfer each wash into the column.	
	11. Rinse container with 10 ml of petroleum ether.	11a. Add the rinse to the column.	
	12. Add 200 ml of the 6% ethyl ether in petroleum ether solution.	12a. Measure in a graduated cylinder. 12b. If prepared just prior to use by the directions given in the reagent preparation section, only 200 ml will be prepared and can be completely transferred to the column. 12c. Add small portion slowly to bring liquid level to the top of the florisil column then add the rest.	
	13. Collect the 200 ml	13a. Close the column's stopcock before the sulfate layer is exposed to the air.	
	14. Add 200 ml of the 15% ethyl ether-petroleum ether solution.		
	15. Immediately replace the 500 ml K-D flask with another clean one.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Sample Clean-Up (Continued)	16. Collect the 15% eluate.	16a. There will be a small overlap; in this case a small portion of the 6% will be collected in the 15% flask. Again close the stopcock to prevent exposure of sulfate layer to the air.	
	17. Just prior to the sulfate layer being exposed add 200 ml of ethyl ether.	17a. For wastewater or if other pesticides are to be monitored other than those listed in the Interim Primary Regulations, a 50% ethyl ether in petroleum ether elution would be carried out at this time followed by the straight ethyl ether elution.	
	18. Immediately replace the 500 ml K-D flask with another clean one.		
	19. Concentrate the elution volumes in their respective flasks.	19a. Use the procedure under Sample Concentration, (G.3), Steps 1 through 6.	
	20. Remove the flask from the ampul.		
	21. Rinse lower portion of the flask and glass joint.		
	22. Obtain a final volume of 10 ml.		
	23. Stopper the ampul.		
	24. Inject 5 µl of the fractions.	24a. See F.23a.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Sample Analysis	1. Check instrument parameters.	1a. Column Temp. Flow Detector OV-17 & QF1 - 200° C - 60 ml/min. - 220° C OV-210 - 180° C - 70 ml/min. - 200° C OV-1 - 180° C - 70 ml/min. - 200° C QF1 - SE30 - 200° C - 60 ml/min. - 220° C 1b. These conditions should be monitored by the analyst during operation. 1c. If the instrument was off, turn on and allow to equilibrate overnignt.	
	2. Inject 5 µl of standard mixture 2.	2a. Reagent Preparation Section.	
	3. Determine if response has changed for the standards.	3a. Check retention times as well as peak heights for the known concentrations.	
	4. Inject 5 µl of the method blank.	4a. Continue the chromatogram until the retention time for the last peak has passed.	
	5. Determine if impurities are present which will interfere.		
	6. Inject 5 µl of the sample.	6a. Time the retention time; from first sign of the solvent until the top of the peak, for each peak.	
	7. Compare with standard curve (Step I.2.).	7a. Retention times and numbers of peaks will indicate further actions.	VII.1.7.7a (p. 47)
	8. Inject 5 µl of a known standard mixture that is very close to that of the sample.	8a. Measure the peak area or peak height in millimeters.	
	9. Calculate the amounts of the pesticides present.	9a. See the Calculation Section.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Calculations 1. Lauric Acid Value	1. Use the following steps to calculate the lauric acid value.	1a. The calculation formula is Lauric Acid Value = $\frac{\text{mg lauric acid}}{\text{gm florisil}}$ = 200 - (ml required for titration x $\frac{\text{mg lauric acid}}{\text{ml 0.05N NaOH}}$) the $\frac{\text{mg lauric Acid}}{\text{ml 0.05N NaOH}}$ comes from the standardization of the NaOH (See Section on Standardization of Sodium Hydroxide).	
2. Amount of Florisil to be Used in the Column	1. Calculate the amount of florisil to be used in the columns.	1a. The calculation formula is Amount of florisil to be used = $\frac{110}{\text{Lauric Acid Value}}$ x 20 grams	
		1b. The 110 value is a value abritrarily assigned as the desired adsorptive capacity.	Mills, P.A., Variations of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and its Use in Standardizing Florisil Columns; JAOAC, 51 29 (1968)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Calculations (Continued) 3. Calculation of Results	1. Calculate the micrograms of pesticide per liter of sample.	1a. The calculation formula is $\frac{\text{micrograms}}{\text{liter}} = \frac{A \times B \times V_t}{V_i \times V_s}$ where $A = \frac{\text{ng standard}}{\text{standard area}}$ (obtained in Sample Analysis Section, Step 8) B = Sample Aliquot Area (obtained in Sample Analysis Section, Step 7) V_t = Volume of total extract (i.e. the volume to which the extract was concentrated) in microliters V_i = Volume of extract injected in microliters V_s = Volume of water (sample) extracted in milliters.	

WATER MONITORING PROCEDURE: Determination of Chlorinated Hydrocarbon Pesticides

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analyses
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked. These headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.12	When the Interim Primary Drinking Water Regulations were promulgated they contained the requirement that all Public Water Supplies be monitored for pesticide contamination. Certain monitoring frequencies and analytical methods were prescribed. A level beyond which public notification and other steps were to take effect was set and termed the "Maximum Contaminant Level" (MCL). The MCL's for the Chlorinated Hydrocarbon Pesticides are as follows:	

Endrin	0.0002 mg/liter
Lindane	0.004 mg/liter
Methoxychlor	0.1 mg/liter
Toxaphene	0.005 mg/liter.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.1	Use of high grade carrier gases are recommended. However, occasionally bad cylinders of the Argon-Methane gas can be obtained. Before attaching to the instrument, a slight sniff of the gas should be taken; if a "fishy" ordor is noted, the tank may be contaminated. Use of the gas will produce an off scale peak and very noisy base line. If contaminated gas is used in the instrument, remove from use as soon as it is determined and replace all traps and purge with a good gas.	
C.6.b	OV-210 may be substituted for the QF-1. The OV-210 is a purified version of the QF-1 and does not bleed as much as the QF-1.	
D.1.3.a	Because of the many variables inherent in gas chromatographs, the column packing, column oven temperature and carrier gas flow rate may have to be adjusted to different settings than those given. The analyst should strive to reproduce the retention times given in the body of the paper as guides. The two things which must be obtained are reproducibility and resolution. When these are adequate the system is suitable.	
D.3.2	The analyst must know what the detection limit is in his procedure. The concentrations are so low in drinking water that frequently the results will be non-detectable. In this case the analyst should express the value as "non-detectable," below the detection limit of This detection limit must be at least below the Maximum Contaminant Level for the compound as listed in the Interim Primary Drinking Water Regulations.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
D.4	Lindane, Endrin, and Methoxychlor are quantitated using one peak for each. However, toxaphene has many peaks and should be quantitated by averaging the peak height (in millimeters) of as many peaks as possible. Use only those peaks which are identical in the standard and sample by retention and peak height ratio. The more peaks used in this average, the closer this number will come to the true value. However, when other chlorinated hydrocarbons are present the peaks of toxaphene in areas not affected by the other chlorinated hydrocarbons should be averaged. From running the standards separately these areas can be found. When the peaks of the other chlorinated hydrocarbons are influenced by the presence of toxaphene they should be separated by another technique, i.e. another absorption column or liquid chromatography etc.	
F.1	Different batches of florisil have varying adsorptive capacities. In order to obtain elution of the various pesticides in the same fractions, this adsorptive capacity must be known. A rapid method for determining this adsorptive capacity is to measure the amount of lauric acid adsorbed from hexane solution by a measured amount of florisil. This is referred to as the lauric acid value.	
F.2	This procedure need be carried out only once with each batch of florisil. As a new supply is purchased, the lauric acid value and this procedure should be determined again, using this same procedure.	
G.2.1f	Samples up to 3 liters can be extracted to increase the sensitivity. However, larger volumes of extraction solvent (methylene chloride-hexane) will be needed. Use 100 ml portions in place of the 60 ml. The separatory funnel size will also need to be increased to a 6 liter size.	
G.3.16	Samples containing small quantities of pesticides (low nanogram amounts) are concentrated to 1 ml. Should the concentrations allow, it is possible to concentrate to 10 ml for higher values. In this case the initial concentration with the Snyder Column will reduce the volume to 5 to 6 ml and the	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
G.3.16 (Continued)	walls of the flask and glass joint can be washed to give a final volume of 10 ml.	
I.7.7a	Major peaks not matching those of the standards along with increased peak heights on some peaks will indicate the presence of interfering compounds. If this is the case, the florisil column clean-up should be used. If no interferences are present, identify and quantitate the peaks.	

TABLE 3

RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase[1]	1.5% OV-17 + 1.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temp.	200° C	180° C	180° C	200° C
Argon/Methane Carrier Flow	60 ml/min.	70 ml/min.	70 ml/min.	60 ml/min.
Pesticide	RR	RR	RR	RR
Lindane	0.69	0.81	0.44	0.60
Aldrin	1.00	1.00	1.00	1.00
Endrin	2.93	3.56	2.18	2.42
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min absolute)	3.5	2.6	4.0	5.6

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[1] All columns glass, 180 cm x 4 mm ID, solid support Gas-Chrom Q (100/120 mesh)

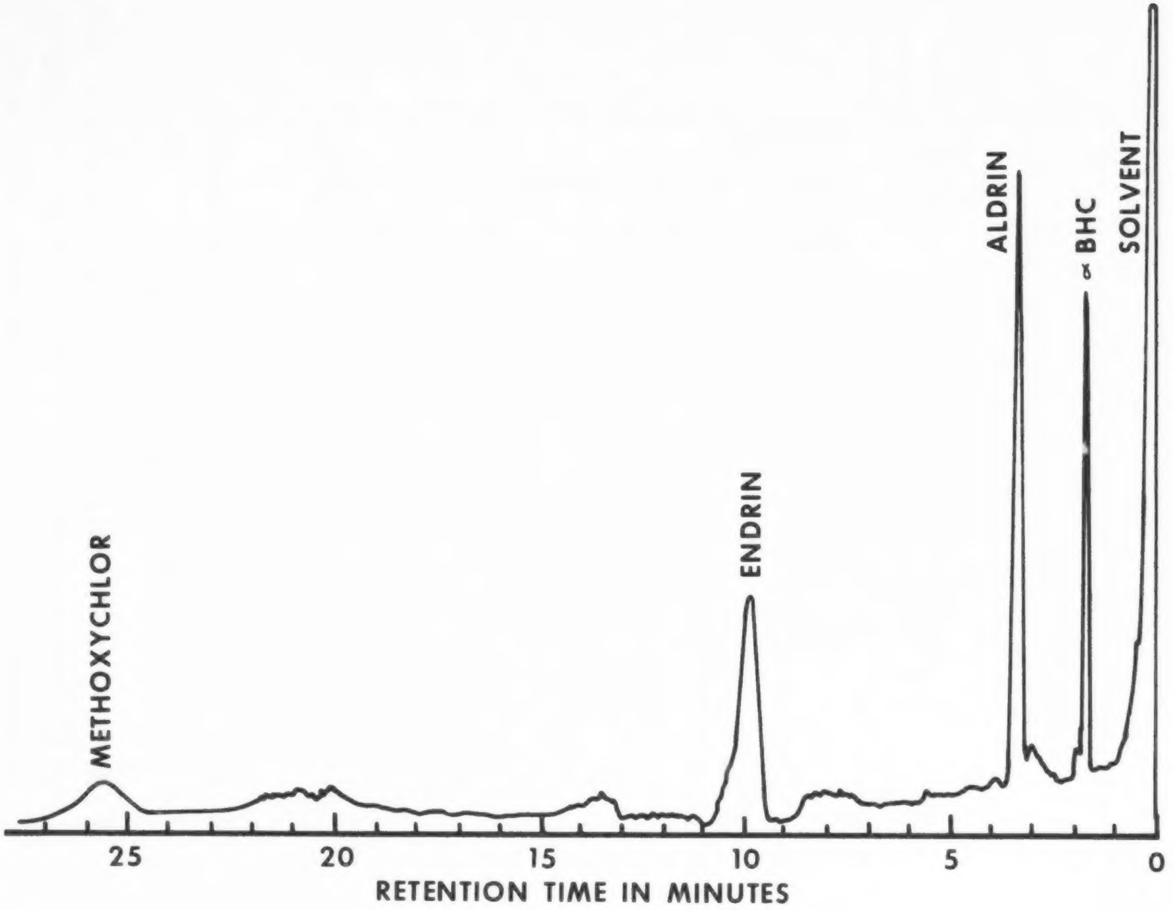

FIGURE 1. COLUMN PACKING: 1.5% OV-17 + 1.95% QF-1, CARRIER GAS: ARGON/METHANE AT 60 ML/MIN, COLUMN TEMPERATURE: 200 C, DETECTOR: ELECTRON CAPTURE.

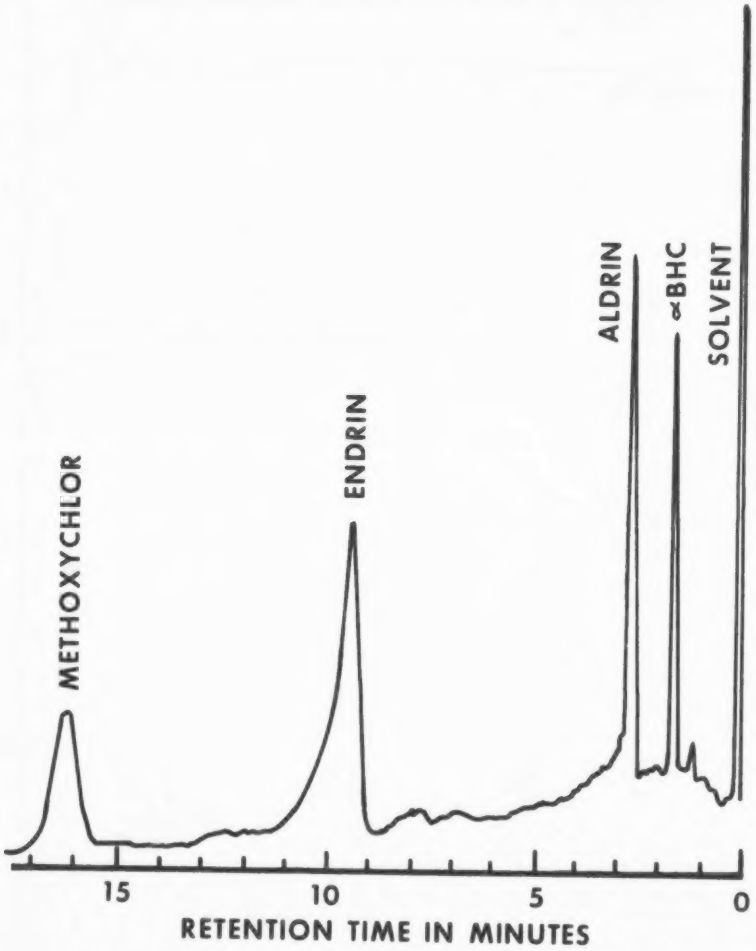

FIGURE 2. COLUMN PACKING: 5% OV-210, CARRIER GAS: ARGON/METHANE AT 70 ML/MIN, COLUMN TEMPERATURE: 180 C, DETECTOR: ELECTRON CAPTURE.

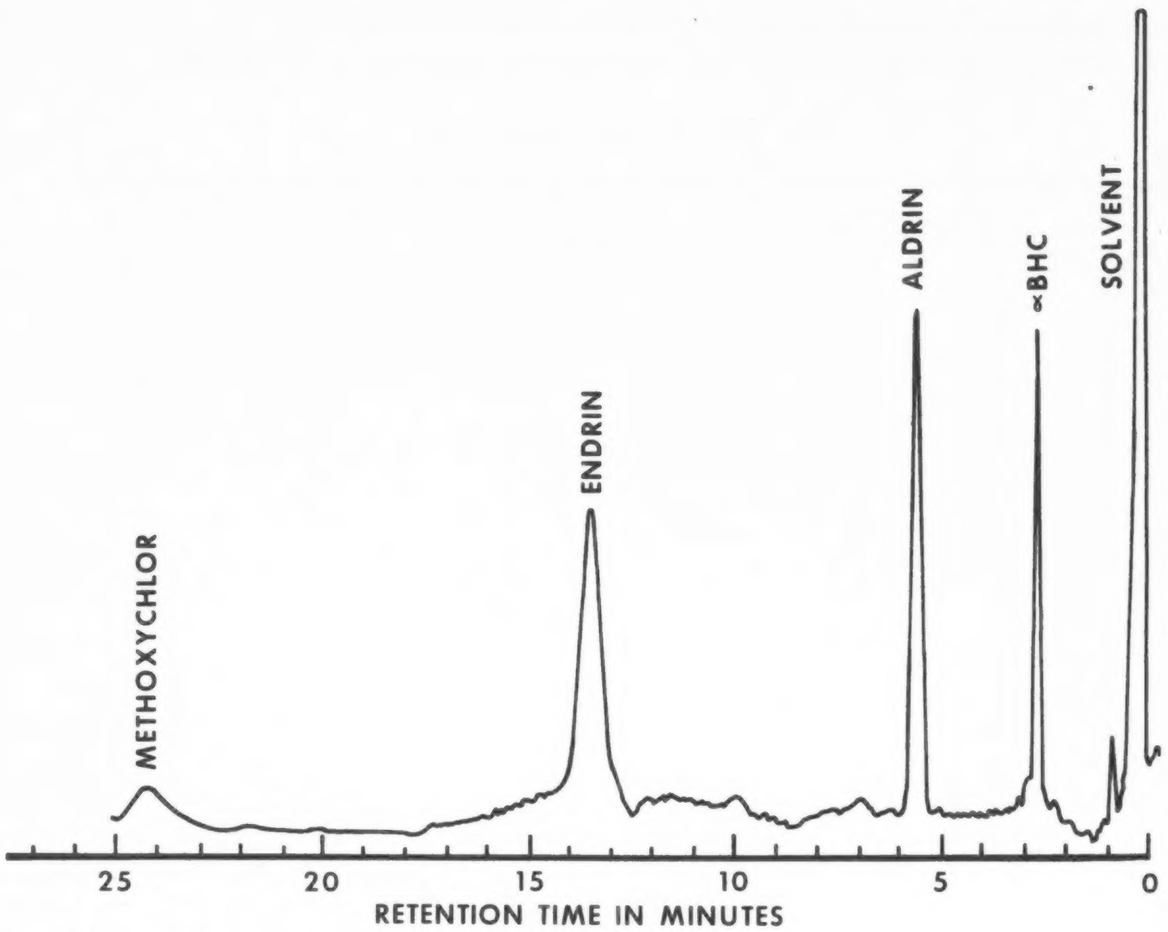

FIGURE 3. COLUMN PACKING: 6% QF-1 + 4% SE-30, CARRIER GAS: ARGON/METHANE AT 60 ML/MIN, COLUMN TEMPERATURE: 200 C, DETECTOR: ELECTRON CAPTURE.

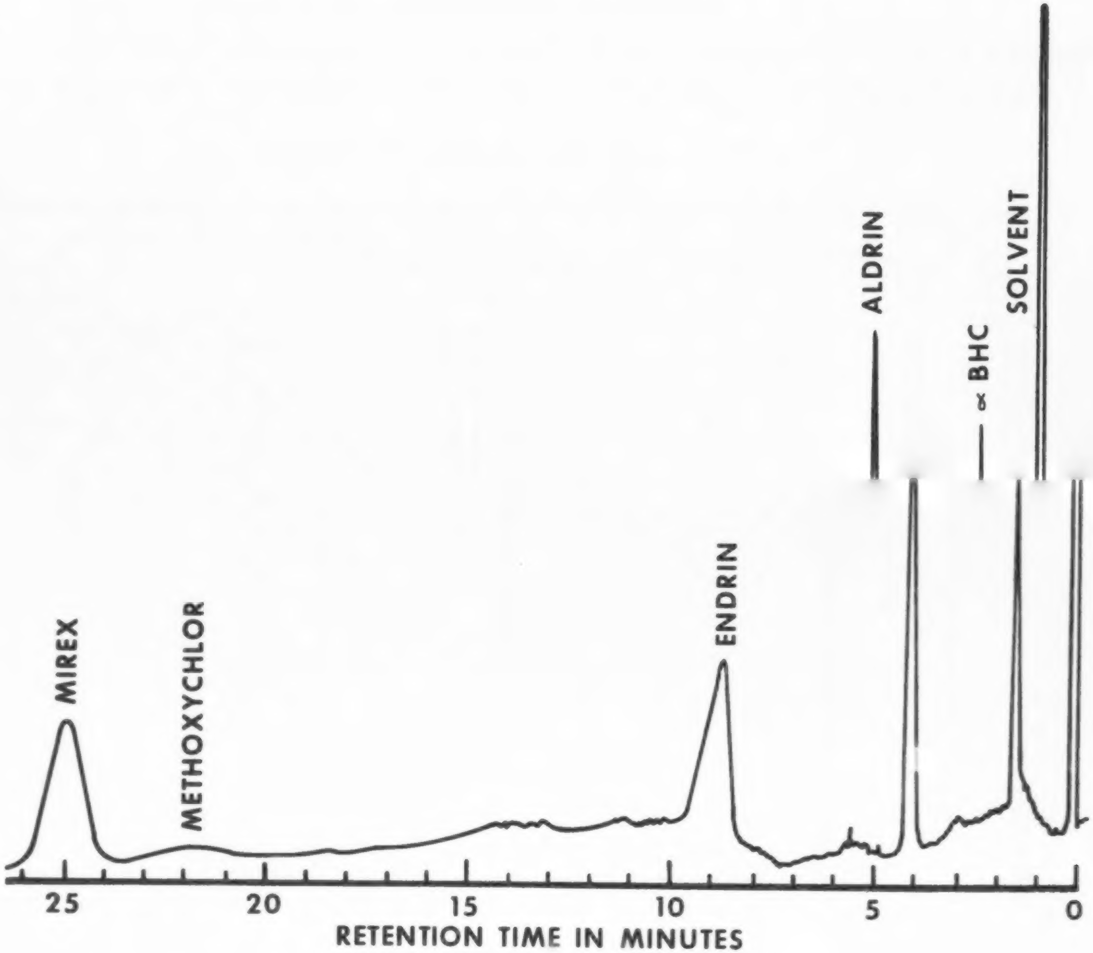

FIGURE 4. COLUMN PACKING: 3% OV-1, CARRIER GAS: ARGON/METHANE AT 70 ML/MIN, COLUMN TEMPERATURE: 180 C, DETECTOR: ELECTRON CAPTURE.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF CHLORINATED PHENOXY ACID HERBICIDES

as applied in

POTABLE WATER TREATMENT FACILITIES

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

CH.PES.lab.WMP.1.11.77

WATER MONITORING PROCEDURE: Determination of Chlorinated Phenoxy Acid Herbicides

Operational Procedures

A. Glassware Preparation

B. Reagent Preparation

C. Standard Preparation

D. Instrument Set-Up

1. Gas Connection
2. Detector Installation
3. Carrier Gas Leak Check
4. Column Conditioning

E. Instrument Calibration

1. Optimization
2. Retention Times
3. Detection Limits

F. Sample Treatment

1. Pre-Treatment
2. Hydrolysis
3. Esterification

G. Sample Analysis

H. Calculations

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WATER MONITORING PROCEDURE: Determination of Chlorinated Phenoxy Acid Herbicides

Equipment and Supply Requirements

A. Capital Equipment:

1. Gas chromatograph equipped with
   - a. Glass lined injection port
   - b. Electron capture detector - tritium or nickel 63
   - c. Recorder - potentiometric strip chart (10 in.-25 cm) compatible with the detector
2. Gas chromatographic column (best purchased from gas chromatographic supply house)
   - a. Tubing - Pyrex (180 cm long (6 ft.) x 4 mm ID)
   - b. Glass wool - silanized
   - c. Solid support - gas chrom Z (100-120 mesh)
   - d. Liquid phase - expressed as weight percent coated on solid support
     - 1) OV-210, 5%
     - 2) OV-17, 1.5% plus QF-1, 1.95%
3. Hot water bath - capable of keeping temperature at 50°-100° C
4. Source of high quality distilled water
5. Rotometers - If the instrument is not equipped with meters to monitor the flows of gases, these should be purchased as options.
6. Analytical balance - with a 0.1 milligram sensitivity
7. Trip or platform balance - with a 0.1 or 0.01 gram sensitivity
8. Oven - capable of maintaining 130° C
9. Stop watch - capable of measuring at least 1/2 hour, the 60 second cycle divided to 1/5 second
10. Cylinder of Argon-methane (95 + 5%) for use with pulsed mode detector OR Nitrogen - purified grade, moisture and oxygen free, for use with a DC mode detector
11. Pressure regulator - two stage with a CGA 580 fitting for nitrogen or a CGA 350 fitting for Argon-methane
12. Filter - for carrier gas, molecular sieve type
13. Micro syringes - 5, 10, 50 μl sizes
14. pH meter (optional)

Equipment and Supply Requirements (Continued)

15. Desiccator
16. Muffle furnace (optional) - capable of heating to 400° C
17. Source of Vacuum
18. Trap for oxygen

B. Reusable Supplies:

1. Beaker - 100 ml, 1/sample, duplicate, blank, standard
2. Cylinders, graduated

   2, 25 ml
   2, 100 ml
   1, 250 ml
   1, 1000 ml

3. Cylinder, graduated - glass-stoppered

   1-25 ml/sample, duplicate, blank, standard

4. Flasks, Erlenmeyer

   1, 125 ml/sample, duplicate, blank, standard
   1, 250 ml/sample, duplicate, blank, standard
   1, 1000 ml/sample, duplicate, blank, standard
   Erlenmeyer - glass-stoppered
   1, 250 ml/sample = 𝕊 19/22

5. Flasks - volumetric

   4, 100 ml
   6, 10 ml

6. Funnel, 1 - 50 mm diameter top/sample, duplicate, blank, standard
7. Funnel - separatory (with Teflon stopcock)

   1, 2000 ml/sample, duplicate, blank, standard
   1, 60 ml/sample, duplicate, blank, standard

8. Glass stirring rod, about 10 cm long
9. Glassware brush

Equipment and Supply Requirements (Continued)

10. Kuderna-Danish (K-D), order from Kontes Glass Corporation

    5 plus 2/sample, concentration ampul, 10 ml calibrated $ 19/22 female
        #K5-70050, size 1025
        1/sample, Snyder col., three section, 150 mm long, #K503000, size 121
        1/sample, Snyder col., one section, #K569001, size 1-19
        1/sample, flask, 250 ml size, #K570001
    About 6/pr., springs, 2/set-up, #K662750
    1/ampul, stoppers for concentrator ampul, #K850500, size 19/22

11. Pipets

    1 box, Pasteur, disposable (140 mm long x 5 mm ID)
    4 - graduated, 1 ml
    5 - volumetric, 1 ml
    3 - volumetric, 2 ml
    2 - volumetric, 10 ml

12. Pipet bulb

    3 ml size for Pasteur type pipets
    Rubber type for pipets

13. Reagent bottles (glass-stoppered, storage)
    5 - 150 ml size
    1 - 500 ml size
    1 - 1000 ml size

14. Rack for separatory funnel

15. 3 - ring stand and clamp

16. 1 ruler - divides in millimeters

17. Safety glasses

18. 1 timer (60 min.)

C. Consumable Supplies

1. 1 box aluminum foil

2. 1 bottle - boiling stones (rinse with hexane)

3. 1 box detergent

4. 25 liters distilled water

5. 1 box glass wool (filtering grade, acid washed)

Equipment and Supply Requirements (Continued)

6. 1 pack graph paper (arithmetic, 10 x 20)
7. 1 roll pH paper (for acid pH)
8. 12 labels
9. 1 note book (bound)
10. 1 pencil or pen
11. 12 weighing boats, plastic, disposable
12. 1 bottle soap solution (any liquid soap mixed with water)
13. Chart paper for records
14. Reagents
    a. Acetone - ACS grade
    b. Alcohol - ethenol, 95%, ACS grade
    c. Benzene - nanograde, distilled in glass
    d. Borontrifluoride - methanol, esterification reagent, 14% BF_3 by weight*
    e. Ethyl ether - nanograde, distilled in glass
    f. Florisil - pesticide residue grade (60-100 mesh), purchase activated at 1250° F and store at 130° C
    g. Herbicide standards, reference grade
    h. Hexane, nanograde, distilled in glass
    i. Potassium hydroxide (KOH), ACS grade
    j. Potassium iodide (KI), ACS grade
    k. Sodium sulfate, ACS, granular
    l. Sulfuric acid, ACS, concentrated

*Available already prepared from: Applied Sicence Laboratories
PO Box 440
State College, PA 16501

WATER MONITORING PROCEDURE: Determination of Chlorinated Phenoxy Acid Herbicides

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Glassware Preparation	1. Clean all glassware.		
	2. Wash with soap and water.		
	3. Rinse with tap water.	3a. At least 10 times.	
	4. Rinse with distilled water.	4a. At least 10 times.	
	5. Muffle at 400° C for 15 to 30 minutes.	5a. Volumetric glassware should not be muffled. 5b. Plastic ware and cap liners for sample containers should not be muffled. 5c. The glassware may be rinsed with redistilled acetone followed by a rinse with pesticide quality hexane in place of the muffling.	
	6. Cool to room temperature.		
	7. Store until used.	7a. Store inverted or cover mouth with aluminum foil. 7b. Sample containers should be stored capped.	
B. Reagent Preparation 1. Distilled Water	1. Distill water.	1a. Use an all glass still. 1b. Extract a volume of distilled water, equal to the sample size used, to check purity. This reagent blank should be analyzed with each set of samples.	
2. Sulfuric Acid (25%) (H_2SO_4)	1. Add 50 ml of water to a 100 ml graduated cylinder.		
	2. Add 25 ml of concentrated sulfuric acid.	2a. Measure in a 25 ml graduated cylinder. 2b. CAUTION: Temperature will rise.	
	3. Cool to room temperature.		
	4. Add water to the 100 ml mark.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	5. Transfer to a reagent bottle.		
	6. Store in freezer section of a refrigerator.		
3. Sodium Sulfate Solution (5%) (Na_2SO_4)	1. Weigh out 5.0 grams of sodium sulfate.	1a. Use a trip balance and a plastic weighing boat. 1b. This is not the acidified sodium sulfate (B.8).	
	2. Transfer to a glass-stoppered reagent bottle.	2a. A 150 ml size.	
	3. Measure out 100 ml water.	3a. In a graduated cylinder.	
	4. Use a small amount of water to wash the weighing boat.	4a. Add washing to reagent bottles.	
	5. Add remaining water to a reagent bottle.		
	6. Mix and label.		
4. Potassium Iodide (10%) (KI)	1. Weigh out 10.0 grams of potassium iodide.	1a. On a trip balance in a plastic weighing boat.	
	2. Transfer to a glass-stoppered reagent bottle.	2a. A 150 ml size.	
	3. Measure out 100 ml water.	3a. In a graduated cylinder.	
	4. Use small amount of water to wash weighing boat.	4a. Add wash to reagent bottle.	
	5. Add remianing water to reagent bottle.		
	6. Mix and label.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued) 5. Potassium Hydroxide (w:v) (KOH)	1. Weigh out 37.0 grams of potassium hydroxide pellets.		
	2. Transfer to a glass-stoppered reagent bottle.	2a. A 150 ml size.	
	3. Measure out 100 ml water.	3a. In a graduated cylinder.	
	4. Use small amount of water to wash the weighing boat.	4a. Add washing to reagent bottle.	
	5. Add remaining water to reagent bottle.	5a. CAUTION: Temperature will rise.	
	6. Mix and label.		
6. Diethyl Ether	1. Test for peroxides.	1a. Purchase distilled in glass or nanograde. 1b. Ether must contain 2% alcohol and be free of peroxides as follows.	
	2. Rinse a 25 ml glass-stoppered graduated cylinder with ethyl ether.	2a. Discard the rinse.	
	3. Add 10 ml ether to the cylinder.		
	4. Add 1 ml freshly prepared potassium iodide (KI) solution.	4a. Use a 1.0 ml volumetric pipet.	
	5. Mix by inverting.	5a. Two or three times. Open stopper to allow pressure out.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	6. Let stand one minute.		
	7. No yellow color should be observed in either layer.	7a. If yellowing occurs, the peroxides can be decomposed by adding 40 ml of 30% ferrous sulfate solution to each liter of ether. (CAUTION: Reaction may be violent if ether contains a high concentration of peroxides). Then distill. 7b. If this is needed, an alternate source for a better product should be sought.	
	8. Discard tested ether.		
	9. Add 20 ml of 95% ethanol to one liter of ether.		
	10. Store in refrigerator.	10a. Storage of all flammable solvents should be in an explosion proof refrigerator. 10b. Store in reagent bottle glass-stoppered (1 liter size).	
7. Ethyl Ether - Hexane Mix (1:1)	1. Mix equal amounts of the hexane and ethyl ether.	1a. Example: Add 250 ml hexane to 250 ml ether.	
	2. Store in glass-stoppered reagent bottles.	2a. Use a 500 ml size.	
8. Sodium Sulfate (Acidified)	1. Weigh out 100 grams of sodium sulfate.	1a. Use a trip balance. 1b. Use a 250 ml Erlenmeyer flask.	
	2. Add ether to just cover the sodium sulfate.		
	3. Add 0.1 ml of concentrated sulfuric acid.	3a. Use caution when working with concentrated acids. 3b. Use safety glasses.	
	4. Swirl to mix.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)	**5. Remove ether with vacuum.**		
	6. Weigh 1.0 gram of the dried sodium sulfate.	6a. On a trip balance. 6b. In a 50 ml beaker.	
	7. Add 5 ml of distilled water.	7a. Use a 5 ml graduated pipet.	
	8. Swirl to mix.		
	9. Check pH of the solution.	9a. Solution should have a pH below 4. 9b. If not, repeat steps 2 through 9. 9c. Use a pH meter or paper that can distinguish a pH below 4.	
	10. Discard the solution.	10a. Prepared in step 9.	
	11. Store the remaining sodium sulfate at 130° C in glass bottles in an oven.	11a. Or activate overnight at 130° C before use, storing in a desiccator.	
C. Standard Preparation 1. Stock 2,4 Dichlorophenoxyacetic acid (2,4-D)	1. Weigh out 0.100 grams of 2,4-D.	1a. Prepare standard as shown in the accompanying chart. 1b. Weigh on an analytical balance. 1c. Use a plastic weighing boat. 1d. Carry out this procedure in a hood or well ventilated area.	I.C.1.1a (p. 36)
	2. Measure out 60 ml of ethyl ether.	2a. In a 100 ml graduated cylinder. 2b. CAUTION: Ether is extremely flammable.	
	3. Pour 40 ml of ethyl ether into a glass-stoppered 100 ml volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standard Preparation (Continued)	4. Transfer the 2,4-D into the volumetric flask.		
	5. Use the remaining ether to wash the weighing boat.		
	6. Dissolve the 2,4-D.		
	7. Dilute to the mark with hexane.	7a. Solution contains 1 mg/ml.	
	8. Stopper and label.		
2. Stock Silvex	1. Perform steps 1 through 8 above using silvex.	1a. Solution contains 1 mg/ml.	
3. Working Standard 2,4-D	1. Add about 50 ml of the ethyl ether:hexane mixture (reagent 7) to a glass-stoppered 100 ml volumetric flask.	1a. Just estimate this amount.	
	2. Pipet 10.0 ml of the stock 2,4-D into the flask.	2a. Use a 10.0 ml volumetric pipet.	
	3. Dilute to the mark with the ether:hexane mixture.	3a. Solution contains 100 $\frac{\mu g}{ml}$	
	4. Stopper and label.		
4. Working Standard - Silvex	1. Add about 50 ml of the ethyl ether:hexane mixture (reagent) to a glass-stoppered 100 ml volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standard Preparation (Continued)	**2. Pipet 1.0 ml of the stock silvex into the flask.**		
	3. Dilute to the mark with ether:hexane mixture.	3a. Solution contains 10 $\frac{\mu g}{ml}$	
	4. Stopper and label.		
5. Esterification of Standard for Chromatography 2,4-D	**1. Turn on water bath and steam bath.**	1a. Allow water bath to reach 50° C. 1b. Allow steam bath to boil.	VII.5.1 (p. 38)
	2. Pipet 1.0 ml of the working standard into a 10 ml concentrator ampul.	2a. Use a 1.0 ml volumetric pipet. 2b. See equipment section, K-D concentrator ampul.	
	3. Add 0.5 ml of Benzene.	3a. Use a 1 ml graduated pipet.	
	4. Insert a two chamber evaporative column.	4a. See equipment section. 4b. Be sure to attach springs.	
	5. Place assembly into ring stand and support with clamp.		
	6. Place over steam bath.	6a. The height above the steam bath will have to be adjusted to speed up or slow down the evaporation. The analyst should not allow the material to go to dryness.	
	7. Lower volume to 0.4 ml.		
	8. Remove from steam bath and allow to cool.		
	9. Remove the evaporative column.		

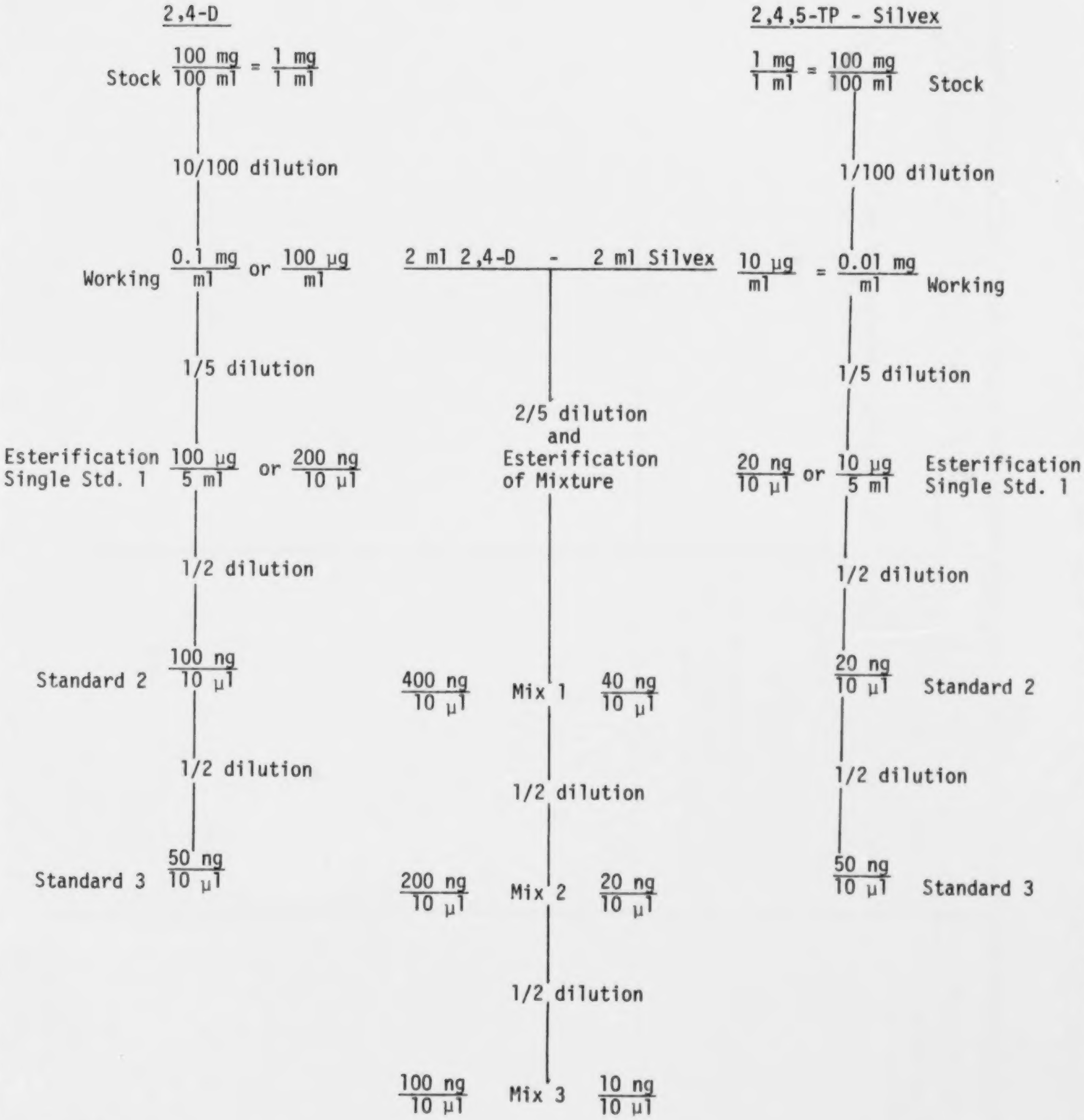
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standard Preparation (Continued)	10. Add 0.5 ml of the borontrifluoride-methanol reagent.		
	11. Replace the evaporative column.		
	12. Lower into a preheated 50° C water bath.	12a. This is not a steam bath. 12b. The water bath should be preheated to 50° C and checked with a thermometer.	
	13. Heat for 30 minutes.		
	14. Remove from water bath.		
	15. Allow to cool to room temperature.		
	16. Remove column.		
	17. Wash walls of concentrator ampul with benzene until a total volume of 5 ml is reached.	17a. Solution contains 200 ng esterified 2,4-D per 10 µl.	
	18. Stopper and mix.		
6. Esterification of Standard for Silvex Chromatography	1. Repeat steps 1-7 above using silvex working standard in place of the 2,4-D. in step 2.	1a. These esterified standards are at the level of the sample containing the MCL's after it has been concentrated. That is the MCL in 5 ml. 1b. Solution contains 40 ng silvex/ 10 µl.	
7. Esterification of Standard Mixture 1	1. Pipet 2.0 ml of each working standard (for 2,4-D. and silvex) into a 10.0 ml concentrator ampul.	1a. Use two 2 ml volumetric pipets.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standard Preparation (Continued)	2. Add 1.0 ml of benzene.	2a. Use a 1 ml graduated pipet.	
	3. Insert a two chamber evaporative column.	3a. Attach springs.	
	4. Place assembly into ring stand and support with clamp.		
	5. Place over steam bath.	5a. The height above the steam bath will have to be adjusted to speed up or slow down the evaporation rate. The analyst should not allow the solution to go to dryness.	
	6. Lower the volume to 1.0 ml.		
	7. Remove from steam bath.		
	8. Remove evaporative column.		
	9. Add 1.0 ml of the boron-trifluoride methanol reagent.		
	10. Replace the evaporative column.		
	11. Lower into a preheated 50° C water bath.		
	12. Heat for 30 minutes.	12a. The bath must be at temperature before lowering the assembly.	
	13. Remove from water bath.		
	14. Allow to cool.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standard Preparation (Continued)	15. Remove evaporative column.		
	16. Wash walls of concentrator ampul until a total volumn of 5.0 ml is reached.	16a. Solution contains 400 ng 2,4-D/10 µl and 40 ng silvex/10 µl.	
	17. Stopper and mix.		
8. Dilutions	1. Prepare dilutions of this mixture labeled Mix 2 and 3 as shown in Table 1.		

STANDARD PREPARATION

TABLE I


2,4-D
Stock $\frac{100\text{ mg}}{100\text{ ml}} = \frac{1\text{ mg}}{1\text{ ml}}$
10/100 dilution
Working $\frac{0.1\text{ mg}}{\text{ml}}$ or $\frac{100\ \mu\text{g}}{\text{ml}}$
1/5 dilution
Esterification Single Std. 1 $\frac{100\ \mu\text{g}}{5\text{ ml}}$ or $\frac{200\text{ ng}}{10\ \mu\text{l}}$
1/2 dilution
Standard 2 $\frac{100\text{ ng}}{10\ \mu\text{l}}$
1/2 dilution
Standard 3 $\frac{50\text{ ng}}{10\ \mu\text{l}}$
2 ml 2,4-D - 2 ml Silvex
2/5 dilution and Esterification of Mixture
$\frac{400\text{ ng}}{10\ \mu\text{l}}$ Mix 1 $\frac{40\text{ ng}}{10\ \mu\text{l}}$
1/2 dilution
$\frac{200\text{ ng}}{10\ \mu\text{l}}$ Mix 2 $\frac{20\text{ ng}}{10\ \mu\text{l}}$
1/2 dilution
$\frac{100\text{ ng}}{10\ \mu\text{l}}$ Mix 3 $\frac{10\text{ ng}}{10\ \mu\text{l}}$
2,4,5-TP - Silvex
$\frac{1\text{ mg}}{1\text{ ml}} = \frac{100\text{ mg}}{100\text{ ml}}$ Stock
1/100 dilution
$\frac{10\ \mu\text{g}}{\text{ml}} = \frac{0.01\text{ mg}}{\text{ml}}$ Working
1/5 dilution
$\frac{20\text{ ng}}{10\ \mu\text{l}}$ or $\frac{10\ \mu\text{g}}{5\text{ ml}}$ Esterification Single Std. 1
1/2 dilution
$\frac{20\text{ ng}}{10\ \mu\text{l}}$ Standard 2
1/2 dilution
$\frac{50\text{ ng}}{10\ \mu\text{l}}$ Standard 3

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Sep-Up 1. Gas Connection	1. Remove cylinder cap from cylinder of carrier gas.	1a. Tank should be chained to wall or lab bench. 1b. Use Argon-methane for pulsed mode detector or use nitrogen for a detector operated in a DC mode. Consult the instrument manufacturer's manual.	V.D.1 (p. 37)
	2. Install the pressure regulator.	2a. The regulator should have a CGA 350 fitting for Argon-methane or a 580 fitting for nitrogen. 2b. Fitting should be tight to prevent leaks.	
	3. Connect the cylinder to the instrument.	3a. Use Teflon tape on all metal threads to prevent leaks. 3b. Polyethylene (1/8" diameter) tubing can be used. 3c. If plastic tubing is used, nylon ferrules should be used with connector fittings.	
2. Detector Installation	1. Install the electron capture detector.	1a. This can be of tritium or nickel 63 type. 1b. Requires license from Atomic Energy Commission. 1c. See the manufacturer's manual on procedures for installation.	
3. Carrier Gas Leak Check	1. Open master valve on instrument.		
	2. Adjust regulator control.	2a. To about 65 psig and allow to stabilize (about 1 minute).	
	3. Close carrier gas control valve on the instrument.		
	4. Turn off master cylinder valve.		
	5. Observe gage on the cylinder.	5a. Pressure should not drop more than a few psig. 5b. If pressure does drop, use soap solution to locate leak. 5c. Correct leak and check again.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Set-Up (Continued)	6. Use soap solution and check for leaks at the injector port and column connections.		
4. Column Conditioning	1. Install packed column in oven by connecting only the column inlet.	1a. These columns may be purchased and meet the specifications as listed under the equipment section. 1b. Column conditioning is essential to eliminate column bleed of packing materials and to provide acceptable analysis. 1c. Do not connect the column to the detector. 1d. If in doubt as to column installation, refer to the manufacturer's manual.	
	2. Start a flow of carrier gas through the detector only.	2a. Use the purge gas line or in a dual column oven by connecting an unpacked column to the detector. 2b. In some systems it may be necessary to temporarily connect the carrier gas to the air or hydrogen inlet in order to get a flow of carrier gas to the detector. The manufacturer's manual should be consulted.	
	3. Begin a low flow of carrier gas through the column.	3a. Less than 60 ml/min. (~40-50). 3b. To remove oxygen and other trapped gasses. 3c. This will be two separate flows. The column should not be connected to the detector.	
	4. Wait 5 minutes.		
	5. Turn on the power to the oven.	5a. Consult manufacturer's manual for location and necessary steps.	
	6. Adjust column temperature to near maximum recommended temperature for the liquid phase being used.	6a. Still no flow of carrier gas through the column. 6b. Column — Max. Temp. °C OV-210 (5%) — 275 OV-17 (1.5%) and QF-1 (1.95%) — 250	V.D.6.6b (p. 37)
	7. Continue heating for 2 hours.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Set-Up (Continued)	8. Reduce temperature to about 40° C below maximum temperature.	8a. See table above (6b).	
	9. Allow column temperature to equilibrate.	9a. Minimum of 30 minutes. 9b. CAUTION: Bleed off of the liquid phase will occur if the column temperature is not fully equilibrated.	
	10. Check carrier gas flow.	10a. At about 50 ml/minute. 10b. If the instrument has not come equipped with rotometers to monitor the flow rate of the carrier gas, this should be purchased as an option. 10c. The pressure regulator on the cylinder should be set at 65 psig. 10d. The electron capture must be installed but the column connection should not be made.	
	11. Allow to remain at temperature and flow for one hour.		
	12. Increase temperature to about 20°C above operating temperature.	12a. This is operating temperature not maximum temperature. 12b. The temperatures would be: 220° C for OV-17 and QF-1 200° C for OV-210	
	13. Continue the same flow of carrier gas.		
	14. Allow to equilibrate for 24 to 48 hours.	14a. CAUTION: Do not exceed maximum recommended temperature. See 6b this section.	
	15. Turn off oven and allow to cool to room temperature.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Instrument Set-Up (Continued)	16. Connect column to the detector.	16a. Check connection with soapy solution.	
	17. Adjust the carrier gas flow to the method flow rate.	17a. 70 ml per minute.	
	18. Turn on oven and adjust to method column temperature.	18a. 185° C.	
	19. Allow the instrument to equilibrate at least one hour.	19a. Preferably overnight.	
E. Instrument Calibration 1. Optimization	1. Check instrument operating conditions after the instrument has been set up.	1a. The oven temperature should be stabilized at the method temperature of 185° C. 1b. The flow rate constant on 70 ml/minute. 1c. The column conditioned. 1d. The system checked for leaks.	
	2. Inject 5 µl of standard mixture.	2a. This standard mixture was prepared from the stock and esterified. 2b. The actual volume of material injected should be kept constant. That is, the same volume for standards and samples. 2c. The standard mixture 2 can be used to optimize the instrument initially and thereafter to monitor its performance.	
	3. Adjust the operating parameters to achieve optimum results.	3a. Best resolution (separation of peaks) and retention times can be achieved by adjusting the column temperature and/or the carrier gas flow rate.	V.E.1.3a (p. 37)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Instrument Calibration (Continued)		3b. Compare results with the standard chromatograms attached (Figure I). 3c. Caution should be taken to allow the instrument to equilibrate after any changes. 3d. Optimum results would include good separation of peaks, good sensitivity, and good reproducibility.	
2. Retention Time Determination	1. Inject 5 µl of standard 1 of 2,4-D and Silvex one at a time.	1a. The individual standards of the 2,4-D and silvex should be run. Not mixtures. 1b. Injection is a technique which must be learned and practiced in order to make accurate and reproducible injections.	VII.E.2.1b (p. 38)
	2. With a stop watch, measure the time elapsed between the first appearance of the solvent peak and the peak of the standard.	2a. This time is called the retention time. 2b. After the retention times of the single standards have been obtained, the mixtures should be chromatographed to determine that no changes in retention times occur.	
	3. Repeat steps 1 and 2 six more times.	3a. At least 7 repeat times should be obtained and the mean value obtained for each peak. 3b. If in subsequent injections the retention times vary signficiantly, the system parameters (E.1.1a-d) should be checked over.	
3. Detection Limit of the Instrument	1. Inject 5 µl of the herbicide standards.	1a. Begin with standard 1 Table 1 of each herbicide and continue with standard 2 and 3. 1b. The standards must be esterified.	
	2. Continue with standards 2 and 3 of the 2,4-D and then silvex and determine the detection limit of each herbicide.	2a. Always inject 5 µl. 2b. The detection limit is that concentration of a standard whose peak is twice the highest peak caused by instrument noise and is run at the most sensitive setting of the instrument.	V.E.3.2b (p. 37)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Instrument Calibration (Continued)	3. The peak height measured in millimeters should be plotted against the known concentrations to produce a standard curve.	3a. The curve produced can be used to select a concentration that produces a response (peak height) close to a unknown sample's peak height. This concentration will be used to calculate the unknown concentration. See the calculation section.	
F. Sample Treatment 1. Pretreatment	1. Blend the sample.	1a. If suspended matter is present. This is usually not required for drinking waters.	
	2. Adjust pH to 2 or below.	2a. Use concentrated sulfuric acid. 2b. Use a pH meter or indicator paper to measure pH. 2c. Usually not necessary for drinking waters.	
2. Extraction	1. Measure out 1 liter of sample.	1a. Experience with the sample source will indicate if smaller volumes of sample can be used. If smaller volumes are used, they should be diluted to one liter before extraction. 1b. A volume of distilled water equal to the sample volume used should be carried through the procedure every time to act as a method blank. 1c. Analyze one duplicate sample with each run as a quality control check. 1d. One ml each of working dilution of 2,4-D and silvex should be diluted to 1 liter and should be carried through the procedure to check on the standard curve and to assure the analyst of proper operation of the wet and instrumental sections of the method.	
	2. Transfer to a 2 liter separatory funnel.	2a. Use a 2 liter funnel in order to have room to shake.	
	3. Add 150 ml of ether to the separatory funnel.	3a. If the sample container has been emptied, use the ether to wash the container and cylinder used for transfer.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)		3b. Measure in a 250 ml graduated cylinder.	
	4. Shake virorously for one minute.	4a. Pressure may build up in the separatory funnel. Invert, with stopper tightly in place, and open stopcock slowly to relieve pressure. Then continue with shaking until one minute is up. Relieve pressure several times during shaking.	
	5. Place in holder and allow phases to separate for at least 10 minutes.	5a. Holder may be a ring and stand or some form of separatory funnel rack. Places should be provided for the blank, duplicate standard and all samples.	
	6. After separation of the phases is completed, drain the water phase into a one-liter Erlenmeyer flask.	6a. If emulsions form and prevent adequate separation, drain the aqueous layer that has separated. Invert the separatory funnel, and shake rapidly. Vent the funnel frequently to prevent excessive pressure buildup. 6b. Assure no water phase remains with the ether. The water is highly acid and step 7 is to assure an alkaline condition in the ether. 6c. Remove the stopper before opening the stopcock.	
	7. Add 2 ml of the 37% aqueous potassium hydroxide solution (reagent 5) to a 250 ml ground glass stoppered Erlenmeyer flask.	7a. The 5 size of the flask should be capable of fitting the three ball Snyder columns.	
	8. Drain the ether layer from the separatory funnel into the Erlenmeyer containing the potassium hydroxide solution.		
	9. Close the stopcock and pour the aqueous phase into the separatory funnel.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)	10. Add 50 ml of ether.	10a. Use the ether to rinse out the flask used to contain the aqueous layer.	
	11. Stopper and shake vigorously for one minute.	11a. Occasionally invert the separatory funnel and relieve the pressure by slowly opening the stopcock.	
	12. Place in holder and allow phases to separate for at least 10 minutes.		
	13. After separation of the phases is complete, drain the water phase in the one liter Erlenmeyer flask used in step 6.	13a. Assure no water phase remains to be collected with the ether.	
	14. Combine the other phase with the first ether extract.		
	15. Repeat steps 9-14 with a third extraction of 50 ml of ether.		
3. Hydrolysis	1. Add 15 ml of distilled water and one small boiling stone to the flask containing the ether extract.	1a. Use a 25 ml graduated cylinder.	
	2. Insert a 3 section Snyder column into the flask.		
	3. Suspend flask and column over a steam bath.	3a. Support with ring stand and clamp.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)	4. Heat until ether has evaporated.	4a. This should be carried out in a hood. 4b. Do not allow flame in or near the hood. 4c. Ether fumes are extremely flammable.	
	5. Continue heating for a total of 60 minutes.		
	6. Remove and allow to cool.		
	7. Transfer the water concentrate to a 60 ml separatory funnel.	7a. Use the same support as with other separatory funnels.	
	8. Add 20 ml of ether to the separatory funnel.	8a. Use a 25 ml graduated cylinder. 8b. Because the solution is basic, the herbicides will remain in the aqueous phase.	
	9. Stopper and shake one minute.	9a. Vent pressure in separatory funnel.	
	10. Place in holder and allow phases to separate for at least 10 minutes.		
	11. Drain aqueous layer into a 100 ml beaker.		
	12. Drain off and discard the ether.		
	13. Return the water to the separatory funnel.		
	14. Add 20 ml ether and repeat steps 9-13.	14a. Again discard the ether.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)	15. Rinse the 100 ml beaker with a small amount of water and add to the separatory funnel.	15a. Use about 2 ml.	
	16. Add 2 ml of cold (4° C) 25% sulfuric acid (reagent 2) to the separatory funnel.	16a. This reagent should have been stored in the freezer section of a refrigerator (B.2.6). 16b. This will acidify the solution; the herbicides are now soluable in the organic phase.	
	17. Add 20 ml of ether to the separatory funnel.		
	18. Shake one minute.		
	19. Allow to stand and separate for at least 10 minutes.		
	20. Drain the aqueous layer into a 100 ml beaker.		
	21. Add 0.5 grams of acidified anhydrous sodium sulfate (reagent 8) to a 125 ml Erlenmeyer flask.		
	22. Collect the ether in the 125 ml Erlenmeyer flask.	22a. The herbicides are in the ether.	
	23. Return the water layer to the separatory funnel.	23a. Close the stopcock.	
	24. Add 10 ml ether.		
	25. Repeat steps 17-22.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)	26. Extract with another 10 ml volume of ether.	26a. This will be three extractions, one with 20 ml and two with 10 ml of ether. 26b. On final extraction, rinse the 100 ml beaker used to collect the aqueous phase with the ether and then add the ether to separatory funnel.	
	27. Collect all ether extractions in the same 125 Erlenmeyer (step 22).		
	28. Allow the ether extract to remain in contact with the sodium sulfate (step 21) for 2 hours.		
	29. Turn on water bath and heat to 50° C.		
4. Esterification	1. Connect a 10 ml graduated ampul to a 250 ml Kuderna-Danish (K-D) evaporative flask.	1a. Attach springs.	
	2. Clamp flask and ampul to a ring stand.		
	3. Plug the stem of a small funnel with glass wool.	3a. Should have been washed with acid.	
	4. Position the funnel stem into the K-D flask.		
	5. Transfer the ether extract from the Erlenmeyer flask through the funnel and into the K-D flask.	5a. Use a glass stirring rod and crush any caked sodium sulfate. 5b. The sodium sulfate may be transferred to the funnel.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)	6. Wash the Erlenmeyer flask and sodium sulfate with liberal amounts of ether.		
	7. Add 0.5 ml benzene.	7a. Use a 1 ml graduated pipet.	
	8. Position flask above steam bath and evaporate to about 5 ml.	8a. A condenser column is not necessary.	
	9. Rinse the flask with 2 ml of ether.		
	10. Remove ampul from flask.		
	11. Insert the two section Snyder microcolumn into the ampul.	11a. Attach springs.	
	12. Return to steam bath and concentrate to about 0.4 ml.	12a. All ether should have been evaporated.	
	13. Remove from steam bath and allow to cool.		
	14. Remove column.		
	15. Add 0.5 ml of boron trifluoridemethanol reagent.		
	16. Return two chamber column to the ampul.		
	17. Lower into a preheated 50° C water bath.	17a. Support by a ring stand and clamp. 17b. This is not the steam bath.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)	18. Heat for 30 minutes.	18a. The column acts as an air cooled condenser.	
	19. Remove from bath and cool to room temperature.		
	20. Remove column.		
	21. Add enough sodium sulfate solution (reagent 3) to position the interface, between the benzene and aqueous sodium sulfate, into the neck of the K-D ampul.	21a. Usually about 4 to 5 ml.	
	22. Stopper the ampul and shake vigorously for about one minute.		
	23. Allow to stand and separate.	23a. About three minutes.	
	24. Plug a disposable Pasteur pipet with glass wool.		
	25. Add florisil absorbant to the column.	25a. Add enough to provide about 2.0 cm height (in the large diameter section on the pipet).	
	26. Place on top of the florisil some granular sodium sulfate.	26a. Enough to provide a sodium sulfate length of 2.0 cm.	
	27. Support the mini-column.	27a. In a clamp on a stand.	
	28. Position mini-column into the neck of a 10 ml graduated K-D ampul.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Sample Treatment (Continued)	29. Pipet the solvent layer from the neck of the ampul (step 22) to the top of the mini-column.	29a. Another Pasteur pipet and bulb can be used (just the pipet no packing). 29b. Collect as little of the aqueous sodium sulfate as possible.	
	30. Add small amounts of benzene to the ampul, shake, allow to separate and pipet into column.	30a. This is a washing step for the ampul, aqueous phase and column. Little to no water should be transferred to the column. 30b. The final volume will be 5.0 ml. Take care not to wash with volumes of benzene which will surpass this total volume.	
	31. Adjust final volume to 5.0 ml.		
	32. Stopper the ampul.	32a. This extract if kept well stoppered and refrigerated, can be held 30 days.	
G. Sample Analysis	1. Check all instrument parameters.	1a. They should be the same as used to obtain optimum results (section E.1.3).	
	2. Inject 5 μl of the standard mix 2.	2a. Table 1.	
	3. Determine if response has changed.	3a. Compare results for retention times and response (peak height) with those obtained in E.1.2.	
	4. Inject 5 μl of method blank.	4a. No significant peaks should be obtained.	
	5. Inject 5 μl of the standard carried through the procedure.	5a. From section F.2.1.1d.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Sample Analysis (Continued)	6. Inject 5 µl of the sample/s.	6a. Inject 5 µl of the duplicate sample. 6b. Be sure to time the peaks from the first appearance of the solvent peak to the top of each peak.	
	7. Compare with standard curve.	7a. Step E.3.3.	
	8. Choose a standard whose concentration will produce a peak that will approximate that obtained from the sample.		
	9. Calculate the amount of herbicide/s present.	9a. See the calculation section.	
H. Calculations 1. Calculation of Results	1. Calculate the micrograms of methyl ester per liter of sample.	1a. Determine the methyl ester concentration by using the equation below. $\text{micrograms per liter} = \frac{A \times B \times V_t}{V_i \times V_5}$ where $A = \frac{\text{ng standard}}{\text{standard area}}$ (obtained in Sample Analysis section, step G.8) B = Sample aliquot area (step G.6) V_t = Volume of total extract in microliters (the volume to which the extract was concentrated i.e., 5 ml)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Calculations (Continued)		V_i = Volume of extract injected in microliters V_s = Volume of water (sample) extracted in milliliters $\mu g\ acid = \mu g\ ester \times \frac{\text{Molecular Wt. Acid}}{\text{Molecular Wt. Ester}}$ Molecular Weight: 2,4-D (acid) = 222.0 2,4-D (ester) = 236.0 Silvex (acid) = 269.5 Silvex (ester) = 283.5	
	2. Convert to micrograms of acid per liter of sample.		
2. Reporting Results	1. Report results in mg per liter as the acid.	1a. Without correction for recovery data. 1b. Report duplicate and spiked sample results when analyzed.	

WATER MONITORING PROCEDURE: Determination of Chlorinated Phenoxy Acid Herbicides

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

Training Guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

INTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.1.1a	When the National Interim Primary Drinking Water Regulations were promulgated they contained the requirement that all Public Water Supplies be monitored for herbicide contamination. Certain monitoring frequencies and analytical methods were prescribed. A level beyond which public notifications and other steps were to be carried out was set and termed the Maximum Contaminant Level (MCL). The MCL's for the chlorophenoxy herbicides are as follows: 2,4, Dichlorophenoxyacetic acid (2,4-D) - 0.1 mg/liter 2,4,5-Trichlorophenoxypropionic acid (2,4,5-TP) - 0.01 mg/liter These materials are used extensively for weed control in lakes, streams and irrigation canals. Phenoxy acid herbicides are very potent even at low concentrations.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.1.3a	Because of the many variables inherent in gas chromatography, the column packing, column oven temperature and carrier gas flow rate may have to be adjusted to different settings than those given. The analyst should strive to reproduce the retention times given in the body of the paper as guides. The two things which must be obtained are reproducibility and resolution. When these are adequate, the system is suitable.	
E.3.2b	The analyst must determine the detection limit for each herbicide. If a sample is taken through the procedure and no peaks are obtained, while peaks are obtained for a standard carried along with the sample, then the analyst is assured that herbicides are not present in concentrations above his detection limit. Whenever reporting such results the analyst should report the detection limit and state that no herbicide is present above that concentration. The values $\frac{200\ \mathrm{ng}}{10\ \mu\mathrm{l}}$ for 2,4-D and $\frac{20\ \mathrm{ng}}{10\ \mu\mathrm{l}}$ for silvex represent the published MCL for that compound in a 5 ml volume, that is the concentration factor of this procedure.	
D.1	Use of high grade carrier gases are recommended. However, occasionally bad cylinders of the Sigon-Methane gas can be obtained. Before attaching it to the instrument, a slight sniff of the gas should be taken; if a "fishy" smell is noted, the tank may be contaminated. Use of the gas will produce an off-scale peak and very noisy base line. If contaminated gas is used in the instrument, remove from use as soon as detected and replace all traps and purge with a noncontaminated gas.	
D.6.6b	OV-210 may be substituted for QF-1. The OV-210 is a purified version of the QF-1 and does not tend to bleed as much as the QF-1.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
VII.5.1	Standards may be prepared from the acids or the methyl esters. If prepared from the methyl esters, the esterification steps are not necessary. However, the methyl esters are hard to purchase. Since samples must be esterified, the standard should also be esterified. If anything less than 100% conversion of the acid to the ester is obtained in the esterification steps, the standards will not reflect this and an incorrect analytical result can be obtained. Consequently, this procedure has been written using an esterification step for the standards.	

TABLE 2

RETENTION RATIOS FOR METHYL ESTERS OF SOME CHLORINATED PHENOXY ACID HERBICIDES RELATIVE TO 2,4-D

Liquid Phase[1]	1.5% OV-17 + 1.95% QF-1	5% OV-210
Column Temp.	185° C	185° C
Argon/Methane Carrier Flow	70 ml/min.	70 ml/min.
Herbicide	RR	RR
2,4-D	1.00	1.00
Silvex	1.34	1.22
2,4-D (minutes absolute)	2.00	1.62

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[1] All columns glass, 180 cm x 4 mm ID, solid support Gas Chrom Q (100/120 mesh)

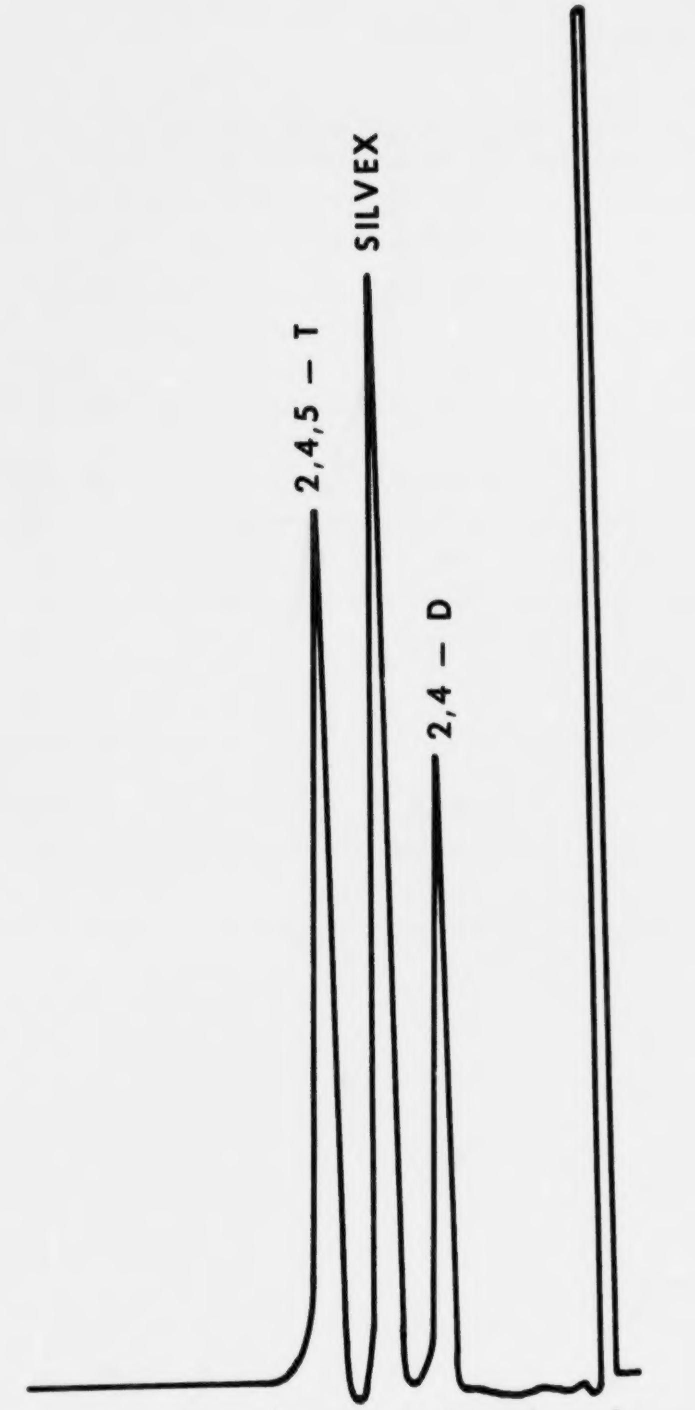

FIG. 1 Column: 1.5% OV–17+1.95% QF–1,
Carrier Gas: Argon (5%) /Methane: 70 ml/min.,
Column Temp. 185 C, Detector: Electron Capture.

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federal register

WEDNESDAY, DECEMBER 24, 1975

THE NATIONAL ARCHIVES OF THE UNITED STATES • 1934 •

PART IV:

ENVIRONMENTAL PROTECTION AGENCY

■

WATER PROGRAMS

National Interim Primary Drinking Water Regulations

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Title 40—Protection of Environment

CHAPTER I—ENVIRONMENTAL PROTECTION AGENCY

SUBCHAPTER D—WATER PROGRAMS

[FRL 464-7]

PART 141—NATIONAL INTERIM PRIMARY DRINKING WATER REGULATIONS

On March 14, 1975, the Environmental Protection Agency (EPA) proposed National Interim Primary Drinking Water Regulations pursuant to sections 1412, 1414, 1415, and 1450 of the Public Health Service Act ("the Act"), as amended by the Safe Drinking Water Act ("SDWA," Pub. L. 93-523), 40 FR 11990. EPA held public hearings on the proposed regulations in Boston, Chicago, San Francisco, and Washington during the month of April. Several thousand pages of comments on the proposed regulations were received and evaluated. In addition, the Agency has received comments and information on the proposed regulations from the National Drinking Water Advisory Council, the Secretary of Health, Education, and Welfare, and from numerous others during meetings with representatives of State agencies, public interest groups and others.

The regulations deal only with the basic legal requirements. Descriptive material will be provided in a guidance manual for use by public water systems and the States.

The purpose of this preamble to the final regulations is to summarize the most significant changes made in the proposed regulations as a result of comments received and the further consideration of available information. A more detailed discussion of the comments and of changes in the proposed regulations is attached as Appendix A.

WATER SYSTEMS COVERED

The Safe Drinking Water Act applies to each "public water system," which is defined in Section 1401(4) of the Act as "a system for the provision to the public of piped water for human consumption, if such system has at least fifteen service connections or regularly serves at least twenty-five individuals." Privately owned as well as publicly owned systems are covered. Service "to the public" is interpreted by EPA to include factories and private housing developments. (See generally, House Report, pp. 16-17.)

The definition of "public water system" proposed in the Interim Primary Drinking Water Regulations sought to explain the meaning of the statutory reference to "regular" service. It was proposed to interpret this term as including service for as much as three months during the year. Because the proposed definition would have excluded many large campgrounds, lodges, and other public accommodations which serve large numbers of tourists but which are open for slightly less than three months each year, the definition in the final version covers systems serving an average of at least twenty-five individuals at least 60 days out of the year. The use of a minimum number of days rather than months also makes clear that a system may qualify as a public water system even if it is not open every day during a given month.

Once "public water system" has been defined, it is necessary to define the two major types of public water systems—those serving residents and those serving transients or intermittent users. The possible health effects of a contaminant in drinking water in many cases are quite different for a person drinking the water for a long period of time than for a person drinking the water only briefly or intermittently. Different regulatory considerations may in some cases apply to systems which serve residents as opposed to systems which serve transients or intermittent users. Accordingly, § 141.2(e) makes clear that all "public water systems" fall within either the category of "community water systems" or the category of "non-community water systems." To make clear which regulatory requirements apply to which type of system, the category covered is specifically indicated throughout the regulations.

The proposed regulations defined a "community water system" as "a public water system which serves a population of which 70 percent or greater are residents." Reliance in the proposed definition on the percentage of water system users who are residents would result in treating some fairly large resort communities with many year-round residents as non-community systems. Therefore, the definition of "community water system" has been changed to cover any system which serves at least 15 service connections used by year-round residents or serves at least 25 year-round residents.

SMALL COMMUNITY WATER SYSTEMS

Many community water systems in the country are quite small. Since it is the intention of the Act to provide basically the same level of health protection to residents of small communities as to residents of large cities, and since a number of advanced water treatment techniques are made feasible only by economies of scale, the cost of compliance with the requirements of the Act may pose a serious problem for many small communities. The regulations seek to recognize the financial problems of small communities by requiring more realistic monitoring for systems serving fewer than 1,000 persons. Variances and exemptions authorized by the Act can also assist in dealing with economic problems of small community systems in appropriate cases, at least temporarily. EPA will provide technical assistance on effective treatment techniques which can be used by small systems.

These methods of dealing with the financial problems of some small community systems may not be sufficient in specific instances to make compliance with all applicable regulatory requirements feasible. EPA is commencing a study of potential problems faced by small community systems in meeting applicable requirements under the Act and these regulations, and, if necessary, will make additional adjustments in the Interim Primary Drinking Water Regulations prior to their effective date.

NON-COMMUNITY SYSTEMS

"Non-community systems" are basically those systems which serve transients. They include hotels, motels, restaurants, campgrounds, service stations, and other public accommodations which have their own water system and which have at least 15 service connections or serve water to a daily average of at least 25 persons. Some schools, factories and churches are also included in this category. It is conservatively estimated that there are over 200,000 non-community water systems in the country. However, it should be recognized that while their number is large, they normally are not the principal source of water for the people they serve.

The regulations as proposed would have applied all maximum contaminant levels to non-community systems as well as to community systems. This approach failed to take into account the fact that the proposed maximum contaminant levels for organic chemicals and most inorganic chemicals were based on the potential health effects of long-term exposure. Those levels are not necessary to protect transients or intermittent users. Therefore, the final regulations provide that maximum contaminant levels for organic chemicals, and for inorganic chemicals other than nitrates, are not applicable to non-community systems. An exception was made for nitrates because they can have an adverse health effect on susceptible infants in a short period of time.

Even without monitoring for organic chemicals or most inorganic chemicals, in the initial stages of implementation of the drinking water regulations, monitoring results from tens of thousands of non-community systems could overwhelm laboratory capabilities and other resources. This could delay effective implementation of the regulations with respect to the community systems which provide the water which Americans drink every day. To avoid this result, non-community systems will be given two years after the effective date of the regulations to commence monitoring. In the meantime, non-community systems which already monitor their water are encouraged to continue to do so, and the States are encouraged to take appropriate measures to test or require monitoring for non-community systems that serve large numbers of people.

Of course, non-community systems which pose a threat to health should be dealt with as quickly as possible. The maximum contaminant levels applicable to non-community water systems therefore will take effect 18 months after promulgation, at the same time as levels applicable to community systems. Inspection and enforcement authority will apply to non-community systems at the same time as to community systems.

SANITARY SURVEYS

EPA encourages the States to conduct sanitary surveys on a systematic basis.

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These on-site inspections of water systems are more effective in assuring safe water to the public than individual tests taken in the absence of sanitary surveys. The regulations provide that monitoring frequencies for coliform bacteria can be changed by the entity with primary enforcement responsibility for an individual non-community system, and in certain circumstances for an individual community system, based on the results of a sanitary survey.

MAXIMUM CONTAMINANT LEVELS

Numerous comments were received by EPA on the substances selected for the establishment of maximum contaminant levels and on the levels chosen. Congress anticipated that the initial Interim Primary Drinking Water Regulations would be based on the Public Health Service Standards of 1962, and this Congressional intent has been followed. Comments received on the various levels did not contain new data sufficient to require the establishment of levels different from those contained in the Public Health Service Standards.

WATER CONSUMPTION

The maximum contaminant levels are based, directly or indirectly, on an assumed consumption of two liters of water per day. The same assumption was used in the 1962 Standards. This assumption has been challenged because of instances where much higher water consumption rates occur. EPA's justification for using the two-liter figure is that it already represents an above average water or water-based fluid intake. Moreover, while the factor of safety may be somewhat reduced when greater quantities of water are ingested, the maximum contaminant levels based on the two-liter figure provide substantial protection to virtually all consumers. If, as has been suggested, a water consumption rate of eight liters per day is used as the basis for maximum contaminant level, all of the proposed MCL's would have to be divided by four, greatly increasing the monitoring difficulties, and in some cases challenging the sensitivity of accepted analytical procedures. It could be expected, in such a case, that the maximum contaminant levels would be exceeded to a significant degree, and that specialized treatment techniques would be required to order that the contaminant levels would be reduced. The economic impact of a move in this direction would be enormous. It is not technically or economically feasible to base maximum contaminant levels on unusually high consumption rates.

SAFETY FACTORS

A question was raised about the fact that different safety factors are contained in various maximum contaminant levels. The levels are not intended to have a uniform safety factor, at least partly because the knowledge of and the nature of the health risks of the various contaminants vary widely. The levels set are the result of experience, evaluation of the available data, and professional judgment. They have withstood the test of time and of professional review. They are being subjected to further review by the National Academy of Sciences in connection with development of data for the Revised Primary Drinking Water Regulations.

MCL'S BASED ON TEMPERATURE

A question was also raised as to whether ranges of maximum contaminant levels should be established on the basis of the climate in the area served by the public water system, as was done with fluoride. EPA believes that the use of a temperature scale for fluoride is more appropriate than for other chemicals because of the studies available on the fluoride-temperature relationship and because there is a small margin with fluoride between beneficial levels and levels that cause adverse health effects.

MCL'S DELETED

Three proposed maximum contaminant levels have been eliminated in the final regulations because they are not justified by the available data. One of these is carbon chloroform extract (CCE), which is discussed separately below. The others are the proposed levels for the standard bacterial plate count and cyanide. In the case of the plate count, it is believed that the coliform limits contained in the regulations, combined with the turbidity maximum contaminant level, adequately deal with bacterial contamination. However, EPA continues to believe that the standard plate count is a valid indicator of bacteriological quality of drinking water, and recommends that it be used in appropriate cases in conjunction with the coliform tests as an operational tool.

The proposed maximum contaminant level for cyanide was eliminated because the possibility of cyanide contamination can be effectively addressed only by the use of emergency action, such as under Section 1431 of the Act. EPA's 1969 Community Water Supply Study did not reveal a single instance in which cyanide was present in a water system at a level greater than one-thousandth of the level at which cyanide is toxic to humans.

Available data indicate that cyanide will be present in water systems at toxic levels only in the event of an accident, such as a spill from a barge collision. Maximum contaminant levels are not the appropriate vehicle for dealing with such rare, accidental contamination.

Heptachor, heptachlor epoxide and chlordane have also been removed from the list of maximum contaminant levels at least temporarily in view of the pending cancellation and suspension proceedings under the Federal Insecticide, Fungicide and Rodenticide Act involving those pesticides. When the results of these proceedings are available, EPA will again consider whether maximum contaminant levels should be established for those three pesticides.

SODIUM AND SULFATES

A number of comments were received on the potential health effects of sodium and sulfates. The National Drinking Water Advisory Council has recommended that consideration be given to the monitoring of these constituents, but has not recommended the adoption of maximum contaminant levels because available data do not support the adoption of any specific levels. EPA has requested the National Academy of Sciences to include sodium and sulfates among the contaminants to be studied by NAS, and to include information on the health effects of sodium and sulfates in the report to be made by NAS in December 1976.

Since a number of persons suffer from diseases which are influenced by dietary sodium intake and since there are others who wish to restrict their sodium intake, it is desirable that the sodium content of drinking water be known. Those affected can, by knowing the sodium concentration in their drinking water, make adjustments to their diets or, in extreme cases, seek alternative sources of water to be used for drinking and food preparation. It is recommended that the States institute programs for regular monitoring of the sodium content of drinking water served to the public, and for informing physicians and consumers of the sodium concentration in drinking water.

A relatively high concentration of sulfate in drinking water has little or no known laxative effect on regular users of the water, but transcients using such water sometimes experience a laxative effect. It is recommended that the States institute monitoring programs for sulfates, and that transients be notified if the sulfate content of the water is high. Such notification should include an assessment of the possible physiological effects of consumption of the water.

PCB'S AND ASBESTOS

An interagency comment expressed concern for asbestos and PCB's in the environment and noted the need for at least a monitoring requirement, if not for MCL's, for these contaminants. EPA is also concerned, but for the moment lacks sufficient evidence regarding analytical methods, health effects, or occurrence in the environment to establish MCL's. The Agency is conducting research and cooperating in research projects to develop criteria for establishing needed limits as quickly as possible. A monitoring study on a number of organic chemical contaminants, including PCB's, for which MCL's are not being established at this time, will be contained in an organic chemical monitoring regulation that is being promulgated with these regulations. Regarding asbestos, HEW and EPA are sponsoring a number of studies this year at an approximate cost of $16 million to establish health effects, anayltical methods and occurrence.

POINT OF MEASUREMENT

Other comments on maximum contaminant levels focused on the proposed requirement that such levels be tested at the consumer's tap. Concern was expressed over the inability of the public water system to control potential sources of contaminants which are under the control of the consumer.

The promulgated definition of "maximum contaminant level," § 141.2(d), retains the requirement that the maximum contaminant level be measured at the tap except in the case of turbidity, which should be measured at the point of entry to the distribution system. However, the definition has been expanded to make clear that contaminants added to the water by circumstances under the control of the consumer are not the responsibility of the supplier of water, unless the contaminants result from corrosion of piping and plumbing resulting from the quality of the water supplied. It should be noted, however, that this requirement should not be interpreted as to discourage local, aggressive cross connection control measures.

COLIFORM BACTERIA MCL'S

The promulgated MCL's for coliform bacteria are basically the 1962 Public Health Service Standards, with minor refinements and clarifications. However, further changes may be desirable. For example, the MCL's for the membrane filter analytical method do not resolve the question of how many coliform bacteria are assumed to be present in a single highly contaminated sample. Some laboratories assume an upper limit of 50, while others seek to continue to count individual bacteria to a level of 100 or even higher in a single sample. The upper limit assumed will affect the monthly average which is calculated to determine compliance with the MCL's.

Another question relating to the coliform bacteria MCL's is the matter of possible spurious positive samples. As the regulations are written, all routine samples taken to determine compliance with the MCL's must be counted, regardless of the results of analysis of any check samples that may be taken. The reason for this is that bacterial contamination is often intermittent or transient, and as a result negative check samples taken a day or more after a positive sample cannot demonstrate that the positive result was in error. It may be possible, however, to prescribe a means of dealing with spurious positive results without compromising the integrity of the MCL's.

A third question concerning the MCL's for coliform bacteria is the relationship of monthly averages of coliform bacteria levels to monthly percentages of positive samples. For example, the monthly average MCL for the membrane filter method is violated if the monthly average exceeds one coliform bacterium per sample. However, for purposes of determining whether the monthly-percentage-of-positive-samples MCL is violated, a sample is counted as positive only if it contains more than four coliform bacteria. Thus, it is possible, particularly when a relatively small number of samples is taken, for a system to fail the monthly average MCL even when no single sample taken during the month is out of compliance with the limit.

These and other questions concerning the coliform bacteria MCL's will be reviewed further by EPA. If review indicates that changes in the MCL's are desirable, those changes will be made as soon as possible but within 6 months, in time to take effect at the same time as the initial Interim Primary Drinking Water Regulations.

ORGANIC CHEMICALS

The proposed maximum contaminant levels for organic pesticides, other than the three which are the subject of cancellation and suspension proceedings, have been retained. It is anticipated that additional organic pesticides will be added to the regulations if surveys of pesticides in drinking water being conducted by EPA indicate that this is needed.

The proposed regulations also contained a maximum contaminant level for organic chemicals obtained by the carbon chloroform extract (CCE) method. It was anticipated by Congress that organic chemicals would be dealt with primarily in the Revised Primary Drinking Water Regulations because of the paucity of accurate data on the health effects of various organic chemicals, the large number of such chemicals, uncertainities over appropriate treatment techniques, and the need for additional information on the incidence of specific organic chemicals in drinking water supplies. EPA thought that the CCE standard might provide an appropriate means of dealing with organic chemicals as a class pending action on the Revised Primary Regulations.

The CCE standard was originally developed as a test for undesirable tastes and odors in drinking water. As concern developed over the health effects of organic chemicals, the possibility of using CCE as a health standard rather than an esthetic standard was considered.

As pointed out by numerous comments, CCE has many failings as an indicator of health effects of organic chemicals. To begin with, the test obtains information on only a fraction of the total amount of organic chemicals in the water sampled. Furthermore, there is serious question as to the reliability of CCE in identifying those organic chemicals which are most suspected of adverse health effects. In addition, there are no existing data on which a specific level for CCE can be established on a rational basis. To establish a maximum contaminant level under these circumstances would almost certainly do more harm than good. It could give a false sense of security to persons served by systems which are within the established level and a false sense of alarm to persons served by systems which exceed the level. It also would divert resources from efforts to find more effective ways of dealing with the organic chemicals problem.

EPA believes that the intelligent approach to the organic chemicals question is to move ahead as rapidly as possible along two fronts. First, EPA is adopting simultaneously with these regulations a Subpart E of Part 141, containing requirements for organic chemical monitoring pursuant to Sections 1445 and 1450 of the Act.

The regulations require that designated public water systems collect samples of raw and treated water for submission to EPA for organics analysis. EPA will analyze the samples for a number of broad organic parameters, including carbon chloroform extract (CCE), volatile and non-volatile total organic carbon (VTOC and NVTOC), total organic chlorine (TOCl), ultraviolet absorbancy, and fluorescence. In addition, monitoring will be required for probably 21 specific organic compounds. Selection of the specific compounds has been based on the occurrence or likelihood of occurrence in treated water, toxicity data and availability of practical analytical methods. Laboratory analyses will be used to evaluate the extent and nature of organic chemical contamination of drinking water, to evaluate the validity of the general organic parameters as surrogates for measures of harmful organic chemicals, and to determine whether there is an adequate basis for establishing maximum contaminant levels for specific organics or groups of organics.

Second, EPA is embarking on an intensive research program to find answers to the following four questions:

1. What are the effects of commonly occurring organic compounds on human health?
2. What analytical procedures should be used to monitor finished drinking water to assure that any Primary Drinking Water Regulations dealing with organics are met?
3. Because some of these organic compounds are formed during water treatment, what changes in treatment practices are required to minimize the formation of these compounds in treated water?
4. What treatment technology must be applied to reduce contaminant levels to concentrations that may be specified in the Primary Drinking Water Regulations?

This research will involve health-effects and epidemiological studies, investigations of analytical methodology, and pilot plant and field studies of organic removal unit processes. Some phases of the research are to be completed by the end of this year, while much of the remainder are to be completed within the next calendar year.

As soon as sufficient information is derived from the monitoring program and related research, the Interim Primary Drinking Water Regulations will be amended so that the organic chemicals problem can be dealt with without delay. The monitoring process will be completed within 1 year.

During the interim period, while satisfactory MCL's for organic contamination in drinking water are being developed, EPA will act in specific cases where appropriate to deal with organic contamination. If the EPA monitoring program reveals serious specific cases of contamination, EPA will work with State and local authorities to identify the source and nature of the problem and to

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take remedial action. EPA will also aid the States in identifying additional community water supplies that require analysis.

PUBLIC NOTICE

The public notice requirements proposed in § 141.32 did not distinguish between community and non-community public water systems. They would have required that public notice of non-compliance with applicable regulations be made by newspaper, in water bills, and by other media for all public water systems. These requirements are inappropriate and ineffective in the case of most non-community water systems. Those systems principally serve transients who do not receive water bills from the system and who probably are not exposed significantly to the local media. A more effective approach would be to require notice that can inform the transient before he drinks the system's water, and thereby both warn the transient and provide an incentive to the supplier of water to remedy the violation. Accordingly, Section 141.32 as adopted provides that in the case of non-community systems, the entity with primary enforcement responsibility shall require that notice be given in a form and manner that will insure that the public using the public water system is adequately informed.

The proposed public notice requirements also failed to distinguish between different types of violations of the Interim Primary Drinking Water Regulations. Since the urgency and importance of a notice varies according to the nature of the violation involved, § 141.32 as promulgated seeks to match the type of notice required with the type of violation involved. Written notice accompanying a water bill or other direct notice by mail is required for all violations of the regulations, including violations of monitoring requirements, and for the grant of a variance or exemption. In addition, notice by newspaper and notification to radio and television stations is required whenever a maximum contaminant level is exceeded, or when the entity with primary enforcement responsibility requires such broader notice.

QUALITY CONTROL AND TESTING PROCEDURES

Section 1401(1) of the Act defines "primary drinking water regulation" to include "quality control and testing procedures." The promulgated regulations include testing requirements for each maximum contaminant level, including check samples and special samples in appropriate cases. The regulations also specify the procedures to be followed in analyzing samples for each of the maximum contaminant levels. These procedures will be updated from time to time as advances are made in analytical methods. For example, references to "Standard Methods for the Examination of Water and Wastewater" are to the current, 13th, edition, but these references will be changed to cite the 14th edition when it is available in the near future.

A key element of quality control for public water systems is accurate laboratory analysis. Section 141.28 of the regulations provides that analyses conducted for the purpose of determining compliance with maximum contaminant levels must be conducted by a laboratory approved by the entity with primary enforcement responsibility. EPA will develop as soon as possible, in cooperation with the States and other interested parties, criteria and procedures for laboratory certification. A State with primary enforcement responsibility will have a laboratory certified by EPA pursuant to the prescribed criteria and procedures, and in turn will certify laboratories within the State.

Record-keeping requirements and reports to the State also will assist in quality control efforts.

RECORD-KEEPING

Adequate record-keeping is necessary for the proper operation and administration of a public water system. It is also important for providing information to the public, providing appropriate data for inspection and enforcement activities and providing information on which future regulations can be based. Accordingly, a new § 141.33 has been added to the regulations to require that each public water system maintain records of sample analyses and of actions to correct violations of the Primary Drinking Water Regulations.

ECONOMIC AND COST ANALYSIS

A comprehensive economics study has been made of the Interim Primary Drinking Water Regulations. This study estimates the costs of the regulations, evaluates the potential economic impact, and considers possible material and labor shortages. The results of this analysis are summarized here.

Total investment costs to community water systems to achieve compliance with these regulations are estimated to be between $1,050 and $1,765 million. It is estimated that non-community systems will invest an additional $24 million. The range of the estimate is due to uncertainty as to the design flow that will be used in installing treatment facilities. Systems not in compliance will have to consider sizing their new components to reflect average daily flow conditions, or maximum daily flow conditions in cases where system storage is not adequate.

This investment will be spread over several years. Investor-owned systems will bear about one-fourth of these costs, and publicly-owned systems the remainder. It is not anticipated that systems will have difficulty financing these capital requirements.

In annual terms, national costs are expected to be within the following ranges:

	In millions
Capital costs	$146–247
Operations and maintenance	263–263
Monitoring (routine only)	17– 35
Total	$426–545

Although these aggregate figures are large, most water consumers will not be significantly affected. For those users in systems serving 10,000 persons or more, the average annual treatment cost per capita may increase from less than $1.00 for systems requiring disinfection and lead control, to between $15 to $35 for control of turbidity and heavy metal removal. For systems serving less than 100 persons, the average annual per capita costs of disinfection, lead control and fluoride/arsenic removal are estimated to be between $2.10 and $11.80. However, if turbidity control or heavy metal removal were required in a system of this size then costs are expected to range from $52 to $237 per year per capita. EPA is aware of the serious potential economic impact on users in these small systems. However, the legislative history specifies that the regulations should be based on costs that can be reasonably afforded by large metropolitan or regional systems. Further economic evaluation of these systems is being conducted, and realistic options for these small systems are being reviewed. Options that will be under consideration include less costly treatment technologies; formation of regional systems; and use of alternative water sources. Industrial and commercial users, whether providing their own water or using public systems, are not expected to be significantly affected by these regulations.

Possible constraints to the implementation of the interim primary regulations were examined. Although there will be an increase in demand for chemicals, manpower, laboratories, and construction of treatment facilities, it is not anticipated that any of these factors will be a serious obstacle to implementation of these regulations over a reasonable time frame.

For the reasons given above, Chapter 40 of the Code of Federal Regulations is hereby amended by the addition of the following new Part 141. These regulations will take effect 18 months after promulgation.

(It is hereby certified that the economic and inflationary impacts of these regulations have been carefully evaluated in accordance with Executive Order 11821)

Dated: December 10, 1975.

RUSSELL E. TRAIN,
Administrator.

Subpart A—General

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141.22 Turbidity sampling and analytical requirements.
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Subpart D—Reporting, Public Notification, and Record-keeping

141.31 Reporting requirements.
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141.33 Record maintenance.

AUTHORITY: Secs. 1412, 1414, 1445, and 1450 of the Public Health Service Act, 88 Stat. 1660 (42 U.S.C. 300g-1, 300g-3, 300j-4, and 300j-9).

Subpart A—General

§ 141.1 Applicability.

This part establishes primary drinking water regulations pursuant to section 1412 of the Public Health Service Act, as amended by the Safe Drinking Water Act (Pub. L. 93-523); and related regulations applicable to public water systems.

§ 141.2 Definitions.

As used in this part, the term:

(a) "Act" means the Public Health Service Act, as amended by the Safe Drinking Water Act, Pub. L. 93-523.

(b) "Contaminant" means any physical, chemical, biological, or radiological substance or matter in water.

(c) "Maximum contaminant level" means the maximum permissible level of a contaminant in water which is delivered to the free flowing outlet of the ultimate user of a public water system, except in the case of turbidity where the maximum permissible level is measured at the point of entry to the distribution system. Contaminants added to the water under circumstances controlled by the user, except those resulting from corrosion of piping and plumbing caused by water quality, are excluded from this definition.

(d) "Person" means an individual, corporation, company, association, partnership, State, municipality, or Federal agency.

(e) "Public water system" means a system for the provision to the public of piped water for human consumption, if such system has at least fifteen service connections or regularly serves an average of at least twenty-five individuals daily at least 60 days out of the year. Such term includes (1) any collection, treatment, storage, and distribution facilities under control of the operator of such system and used primarily in connection with such system, and (2) any collection or pretreatment storage facilities not under such control which are used primarily in connection with such system. A public water system is either a "community water system" or a "non-community water system."

(i) "Community water system" means a public water system which serves at least 15 service connections used by year-round residents or regularly serves at least 25 year-round residents.

(ii) "Non-community water system" means a public water system that is not a community water system.

(f) "Sanitary survey" means an on-site review of the water source, facilities, equipment, operation and maintenance of a public water system for the purpose of evaluating the adequacy of such source, facilities, equipment, operation and maintenance for producing and distributing safe drinking water.

(g) "Standard sample" means the aliquot of finished drinking water that is examined for the presence of coliform bacteria.

(h) "State" means the agency of the State government which has jurisdiction over public water systems. During any period when a State does not have primary enforcement responsibility pursuant to Section 1413 of the Act, the term "State" means the Regional Administrator, U.S. Environmental Protection Agency.

(i) "Supplier of water" means any person who owns or operates a public water system.

§ 141.3 Coverage.

This part shall apply to each public water system, unless the public water system meets all of the following conditions:

(a) Consists only of distribution and storage facilities (and does not have any collection and treatment facilities);

(b) Obtains all of its water from, but is not owned or operated by, a public water system to which such regulations apply:

(c) Does not sell water to any person; and

(d) Is not a carrier which conveys passengers in interstate commerce.

§ 141.4 Variances and exemptions.

Variances or exemptions from certain provisions of these regulations may be granted pursuant to Sections 1415 and 1416 of the Act by the entity with primary enforcement responsibility. Provisions under Part 142, *National Interim Primary Drinking Water Regulations Implementation*—subpart E (Variances) and subpart F (Exemptions)—apply where EPA has primary enforcement responsibility.

§ 141.5 Siting requirements.

Before a person may enter into a financial commitment for or initiate construction of a new public water system or increase the capacity of an existing public water system, he shall notify the State and, to the extent practicable, avoid locating part or all of the new or expanded facility at a site which:

(a) Is subject to a significant risk from earthquakes, floods, fires or other disasters which could cause a breakdown of the public water system or a portion thereof; or

(b) Except for intake structures, is within the floodplain of a 100-year flood or is lower than any recorded high tide where appropriate records exist.

The U.S. Environmental Protection Agency will not seek to override land use decisions affecting public water systems siting which are made at the State or local government levels.

§ 141.6 Effective date.

The regulations set forth in this part shall take effect 18 months after the date of promulgation.

Subpart B—Maximum Contaminant Levels

§ 141.11 Maximum contaminant levels for inorganic chemicals.

(a) The maximum contaminant level for nitrate is applicable to both community water systems and non-community water systems. The levels for the other inorganic chemicals apply only to community water systems. Compliance with maximum contaminant levels for inorganic chemicals is calculated pursuant to § 141.23.

(b) The following are the maximum contaminant levels for inorganic chemicals other than fluoride:

Contaminant	*Level, milligrams per liter*
Arsenic	0.05
Barium	1.
Cadmium	0.010
Chromium	0.05
Lead	0.05
Mercury	0.002
Nitrate (as N)	10.
Selenium	0.01
Silver	0.05

(c) When the annual average of the maximum daily air temperatures for the location in which the community water system is situated is the following, the maximum contaminant levels for fluoride are:

Temperature Degrees Fahrenheit	Degrees Celsius	Level, milligrams per liter
53.7 and below	12.0 and below	2.4
53.8 to 58.3	12.1 to 14.6	2.2
58.4 to 63.8	14.7 to 17.6	2.0
63.9 to 70.6	17.7 to 21.4	1.8
70.7 to 79.2	21.5 to 26.2	1.6
79.3 to 90.5	26.3 to 32.5	1.4

§ 141.12 Maximum contaminant levels for organic chemicals.

The following are the maximum contaminant levels for organic chemicals. They apply only to community water systems. Compliance with maximum contaminant levels for organic chemicals is calculated pursuant to § 141.24.

	Level, milligrams per liter
(a) Chlorinated hydrocarbons:	
Endrin (1,2,3,4,10, 10-hexachloro-6,7-epoxy-1,4, 4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8 - dimethano naphthalene).	0.0002
Lindane (1,2,3,4,5,6-hexachlorocyclohexane, gamma isomer).	0.004
Methoxychlor (1,1,1-Trichloro-2, 2 - bis [p-methoxyphenyl] ethane).	0.1
Toxaphene ($C_{10}H_{10}Cl_8$-Technical chlorinated camphene, 67-69 percent chlorine).	0.005

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(b) Chlorophenoxys:	
2,4 - D, (2,4-Dichlorophenoxyacetic acid).	0. 1
2,4,5-TP Silvex (2,4,5-Trichlorophenoxypropionic acid).	0. 01

§ 141.13 Maximum contaminant levels for turbidity.

The maximum contaminant levels for turbidity are applicable to both community water systems and non-community water systems using surface water sources in whole or in part. The maximum contaminant levels for turbidity in drinking water, measured at a representative entry point(s) to the distribution system, are:

(a) One turbidity unit (TU), as determined by a monthly average pursuant to § 141.22, except that five or fewer turbidity units may be allowed if the supplier of water can demonstrate to the State that the higher turbidity does not do any of the following:

(1) Interfere with disinfection;

(2) Prevent maintenance of an effective disinfectant agent throughout the distribution system; or

(3) Interfere with microbiological determinations.

(b) Five turbidity units based on an average for two consecutive days pursuant to § 141.22.

§ 141.14 Maximum microbiological contaminant levels.

The maximum contaminant levels for coliform bacteria, applicable to community water systems and non-community water systems, are as follows:

(a) When the membrane filter technique pursuant to § 141.21(a) is used, the number of coliform bacteria shall not exceed any of the following:

(1) One per 100 milliliters as the arithmetic mean of all samples examined per month pursuant to § 141.21 (b) or (c);

(2) Four per 100 milliliters in more than one sample when less than 20 are examined per month; or

(3) Four per 100 milliliters in more than five percent of the samples when 20 or more are examined per month.

(b)(1) When the fermentation tube method and 10 milliliter standard portions pursuant to § 141.21(a) are used, coliform bacteria shall not be present in any of the following:

(i) more than 10 percent of the portions in any month pursuant to § 141.21 (b) or (c);

(ii) three or more portions in more than one sample when less than 20 samples are examined per month; or

(iii) three or more portions in more than five percent of the samples when 20 or more samples are examined per month.

(2) When the fermentation tube method and 100 milliliter standard portions pursuant to § 141.21(a) are used, coliform bacteria shall not be present in any of the following:

(i) more than 60 percent of the portions in any month pursuant to § 141.21 (b) or (c);

(ii) five portions in more than one sample when less than five samples are examined per month; or

(iii) five portions in more than 20 percent of the samples when five or more samples are examined per month.

(c) For community or non-community systems that are required to sample at a rate of less than 4 per month, compliance with paragraphs (a), (b)(1), or (b)(2) of this section shall be based upon sampling during a 3 month period, except that, at the discretion of the State, compliance may be based upon sampling during a one-month period.

Subpart C—Monitoring and Analytical Requirements

§ 141.21 Microbiological contaminant sampling and analytical requirements.

(a) Suppliers of water for community water systems and non-community water systems shall analyze for coliform bacteria for the purpose of determining compliance with § 141.14. Analyses shall be conducted in accordance with the analytical recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, 13th Edition, pp. 662–688, except that a standard sample size shall be employed. The standard sample used in the membrane filter procedure shall be 100 milliliters. The standard sample used in the 5 tube most probable number (MPN) procedure (fermentation tube method) shall be 5 times the standard portion. The standard portion is either 10 milliliters or 100 milliliters as described in § 141.14 (b) and (c). The samples shall be taken at points which are representative of the conditions within the distribution system.

(b) The supplier of water for a community water system shall take coliform density samples at regular time intervals, and in number proportionate to the population served by the system. In no event shall the frequency be less than as set forth below:

Population served:	*Minimum number of samples per month*
25 to 1,000	1
1,001 to 2,500	2
2,501 to 3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 9,400	10
9,401 to 10,300	11
10,301 to 11,100	12
11,101 to 12,000	13
12,001 to 12,900	14
12,901 to 13,700	15
13,701 to 14,600	16
14,601 to 15,500	17
15,501 to 16,300	18
16,301 to 17,200	19
17,201 to 18,100	20
18,101 to 18,900	21
18,901 to 19 800	22
19,801 to 20,700	23
20,701 to 21,500	24
21,501 to 22,300	25
22,301 to 23,200	26
23,201 to 24,000	27
24,001 to 24,900	28
24,901 to 25,000	29
25,001 to 28,000	30
28,001 to 33,000	35
33,001 to 37,000	40
37,001 to 41,000	45
41,001 to 46,000	50
46,001 to 50,000	55
50,001 to 54,000	60
54,001 to 59,000	65
59,001 to 64,000	70
64,001 to 70,000	75
70,001 to 76,000	80
76,001 to 83,000	85
83,001 to 90,000	90
90,001 to 96,000	95
96,001 to 111,000	100
111,001 to 130,000	110
130,001 to 160,000	120
160,001 to 190,000	130
190,001 to 220,000	140
220,001 to 250,000	150
250,001 to 290,000	160
290,001 to 320,000	170
320,001 to 360,000	180
360,001 to 410,000	190
410,001 to 450,000	200
450,001 to 500,000	210
500,001 to 550,000	220
550,001 to 600,000	230
600,001 to 660,000	240
660,001 to 720,000	250
720,001 to 780,000	260
780,001 to 840,000	270
840,001 to 910,000	280
910,001 to 970,000	290
970,001 to 1,050,000	300
1,050,001 to 1,140,000	310
1,140,001 to 1,230,000	320
1,230,001 to 1,320,000	330
1,320,001 to 1,420,000	340
1,420,001 to 1,520,000	350
1,520,001 to 1,630,000	360
1,630,001 to 1,730,000	370
1,730,001 to 1,850,000	380
1,850,001 to 1,970,000	390
1,970,001 to 2,060,000	400
2,060,001 to 2,270,000	410
2,270,001 to 2,510,000	420
2,510,001 to 2,750,000	430
2,750,001 to 3,020,000	440
3,020,001 to 3,320,000	450
3,320,001 to 3,620,000	460
3,620,001 to 3,960,000	470
3,960,001 to 4,310,000	480
4,310,001 to 4,690,000	490
4,690,001 or more	500

Based on a history of no coliform bacterial contamination and on a sanitary survey by the State showing the water system to be supplied solely by a protected ground water source and free of sanitary defects, a community water system serving 25 to 1,000 persons, with written permission from the State, may reduce this sampling frequency except that in no case shall it be reduced to less than one per quarter.

(c) The supplier of water for a non-community water system shall sample for coliform bacteria in each calendar quarter during which the system provides water to the public. Such sampling shall begin within two years after the effective date of this part. If the State, on the basis of a sanitary survey, determines that some other frequency is more appropriate, that frequency shall be the frequency required under these regulations. Such frequency shall be confirmed or changed on the basis of subsequent surveys.

(d)(1) When the coliform bacteria in a single sample exceed four per 100 milliliters (§ 141.14(a)), at least two consecutive daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency estab-

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lished by the State, until the results obtained from at least two consecutive check samples show less than one coliform bacterium per 100 milliliters.

(2) When coliform bacteria occur in three or more 10 ml portions of a single sample (§ 141.14(b)(1)), at least two consecutive daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency established by the State, until the results obtained from at least two consecutive check samples show no positive tubes.

(3) When coliform bacteria occur in all five of the 100 ml portions of a single sample (§ 141.14(b)(2)), at least two daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency established by the State, until the results obtained from at least two consecutive check samples show no positive tubes.

(4) The location at which the check samples were taken pursuant to paragraphs (d) (1), (2), or (3) of this section shall not be eliminated from future sampling without approval of the State. The results from all coliform bacterial analyses performed pursuant to this subpart, except those obtained from check samples and special purpose samples, shall be used to determine compliance with the maximum contaminant level for coliform bacteria as established in § 141.14. Check samples shall not be included in calculating the total number of samples taken each month to determine compliance with § 141.21 (b) or (c).

(e) When the presence of coliform bacteria in water taken from a particular sampling point has been confirmed by any check samples examined as directed in paragraphs (d) (1), (2), or (3) of this section, the supplier of water shall report to the State within 48 hours.

(f) When a maximum contaminant level set forth in paragraphs (a), (b) or (c) of § 141.14 is exceeded, the supplier of water shall report to the State and notify the public as prescribed in § 141.31 and § 141.32.

(g) Special purpose samples, such as those taken to determine whether disinfection practices following pipe placement, replacement, or repair have been sufficient, shall not be used to determine compliance with § 141.14 or § 141.21 (b) or (c).

(h) A supplier of water of a community water system or a non-community water system may, with the approval of the State and based upon a sanitary survey, substitute the use of chlorine residual monitoring for not more than 75 percent of the samples required to be taken by paragraph (b) of this section, *Provided*, That the supplier of water takes chlorine residual samples at points which are representative of the conditions within the distribution system at the frequency of at least four for each substituted microbiological sample. There shall be at least daily determinations of chlorine residual. When the supplier of water exercises the option provided in this paragraph (h) of this section, he shall maintain no less than 0.2 mg/l free chlorine throughout the public water distribution system. When a particular sampling point has been shown to have a free chlorine residual less than 0.2 mg/l, the water at that location shall be retested as soon as practicable and in any event within one hour. If the original analysis is confirmed, this fact shall be reported to the State within 48 hours. Also, if the analysis is confirmed, a sample for coliform bacterial analysis must be collected from that sampling point as soon as practicable and preferably within one hour, and the results of such analysis reported to the State within 48 hours after the results are known to the supplier of water. Analyses for residual chlorine shall be made in accordance with "Standard Methods for the Examination of Water and Wastewater," 13th Ed., pp. 129–132. Compliance with the maximum contaminant levels for coliform bacteria shall be determined on the monthly mean or quarterly mean basis specified in § 141.14, including those samples taken as a result of failure to maintain the required chlorine residual level. The State may withdraw its approval of the use of chlorine residual substitution at any time.

§ 141.22 Turbidity sampling and analytical requirements.

(a) Samples shall be taken by suppliers of water for both community water systems and non-community water systems at a representative entry point(s) to the water distribution system at least once per day, for the purpose of making turbidity measurements to determine compliance with § 141.13. The measurement shall be made by the Nephelometric Method in accordance with the recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, 13th Edition, pp. 350–353, or "Methods for Chemical Analysis of Water and Wastes," pp. 295–298, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(b) If the result of a turbidity analysis indicates that the maximum allowable limit has been exceeded, the sampling and measurement shall be confirmed by resampling as soon as practicable and preferably within one hour. If the repeat sample confirms that the maximum allowable limit has been exceeded, the supplier of water shall report to the State within 48 hours. The repeat sample shall be the sample used for the purpose of calculating the monthly average. If the monthly average of the daily samples exceeds the maximum allowable limit, or if the average of two samples taken on consecutive days exceeds 5 TU, the supplier of water shall report to the State and notify the public as directed in § 141.31 and § 141.32.

(c) Sampling for non-community water systems shall begin within two years after the effective date of this part.

(d) The requirements of this § 141.22 shall apply only to public water systems which use water obtained in whole or in part from surface sources.

§ 141.23 Inorganic chemical sampling and analytical requirements.

(a) Analyses for the purpose of determining compliance with § 141.11 are required as follows:

(1) Analyses for all community water systems utilizing surface water sources shall be completed within one year following the effective date of this part. These analyses shall be repeated at yearly intervals.

(2) Analyses for all community water systems utilizing only ground water sources shall be completed within two years following the effective date of this part. These analyses shall be repeated at three-year intervals.

(3) For non-community water systems, whether supplied by surface or ground water sources, analyses for nitrate shall be completed within two years following the effective date of this part. These analyses shall be repeated at intervals determined by the State.

(b) If the result of an analysis made pursuant to paragraph (a) indicates that the level of any contaminant listed in § 141.11 exceeds the maximum contaminant level, the supplier of water shall report to the State within 7 days and initiate three additional analyses at the same sampling point within one month.

(c) When the average of four analyses made pursuant to paragraph (b) of this section, rounded to the same number of significant figures as the maximum contaminant level for the substance in question, exceeds the maximum contaminant level, the supplier of water shall notify the State pursuant to § 141.31 and give notice to the public pursuant to § 141.32. Monitoring after public notification shall be at a frequency designated by the State and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

(d) The provisions of paragraphs (b) and (c) of this section notwithstanding, compliance with the maximum contaminant level for nitrate shall be determined on the basis of the mean of two analyses. When a level exceeding the maximum contaminant level for nitrate is found, a second analysis shall be initiated within 24 hours, and if the mean of the two analyses exceeds the maximum contaminant level, the supplier of water shall report his findings to the State pursuant to § 141.31 and shall notify the public pursuant to § 141.32.

(e) For the initial analyses required by paragraph (a)(1), (2) or (3) of this section, data for surface waters acquired within one year prior to the effective date and data for ground waters acquired within 3 years prior to the effective date of this part may be substituted at the discretion of the State.

(f) Analyses conducted to determine compliance with § 141.11 shall be made in accordance with the following methods:

(1) Arsenic—Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," pp. 95–96, Environ-

mental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(2) Barium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 97–98, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(3) Cadmium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 101–103, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(4) Chromium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 105–106, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(5) Lead—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 112–113, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(6) Mercury—Flameless Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," pp. 118–126, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(7) Nitrate—Brucine Colorimetric Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 461–464, or Cadmium Reduction Method, "Methods for Chemical Analysis of Water and Wastes," pp. 201–206, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(8) Selenium—Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," p. 145, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(9) Silver—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater", 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes", p. 146, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(10) Fluoride—Electrode Method, "Standard Methods for the Examination of Water and Wastewater", 13th Edition, pp. 172–174, or "Methods for Chemical Analysis of Water and Wastes," pp. 65–67, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974, or Colorimetric Method with Preliminary Distillation, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 171–172 and 174–176, or "Methods for Chemical Analysis of Water and Wastes," pp. 59–60, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

§ 141.24 Organic chemical sampling and analytical requirements.

(a) An analysis of substances for the purpose of determining compliance with § 141.12 shall be made as follows:

(1) For all community water systems utilizing surface water sources, analyses shall be completed within one year following the effective date of this part. Samples analyzed shall be collected during the period of the year designated by the State as the period when contamination by pesticides is most likely to occur. These analyses shall be repeated at intervals specified by the State but in no event less frequently than at three year intervals.

(2) For community water systems utilizing only ground water sources, analyses shall be completed by those systems specified by the State.

(b) If the result of an analysis made pursuant to paragraph (a) of this section indicates that the level of any contaminant listed in § 141.12 exceeds the maximum contaminant level, the supplier of water shall report to the State within 7 days and initiate three additional analyses within one month.

(c) When the average of four analyses made pursuant to paragraph (b) of this section, rounded to the same number of significant figures as the maximum contaminant level for the substance in question, exceeds the maximum contaminant level, the supplier of water shall report to the State pursuant to § 141.31 and give notice to the public pursuant to § 141.32. Monitoring after public notification shall be at a frequency designated by the State and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

(d) For the initial analysis required by paragraph (a) (1) and (2) of this section, data for surface water acquired within one year prior to the effective date of this part and data for ground water acquired within three years prior to the effective date of this part may be substituted at the discretion of the State.

(e) Analyses made to determine compliance with § 141.12(a) shall be made in accordance with "Method for Organochlorine Pesticides in Industrial Effluents," MDQARL, Environmental Protection Agency, Cincinnati, Ohio, November 28, 1973.

(f) Analyses made to determine compliance with § 141.12(b) shall be conducted in accordance with "Methods for Chlorinated Phenoxy Acid Herbicides in Industrial Effluents," MDQARL, Environmental Protection Agency, Cincinnati, Ohio, November 28, 1973.

§ 141.27 Alternative analytical techniques.

With the written permission of the State, concurred in by the Administrator of the U.S. Environmental Protection Agency, an alternative analytical technique may be employed. An alternative technique shall be acceptable only if it is substantially equivalent to the prescribed test in both precision and accuracy as it relates to the determination of compliance with any maximum contaminant level. The use of the alternative analytical technique shall not decrease the frequency of monitoring required by this part.

§ 141.28 Approved laboratories.

For the purpose of determining compliance with § 141.21 through § 141.27, samples may be considered only if they have been analyzed by a laboratory approved by the State except that measurements for turbidity and free chlorine residual may be performed by any person acceptable to the State.

§ 141.29 Monitoring of consecutive public water systems.

When a public water system supplies water to one or more other public water systems, the State may modify the monitoring requirements imposed by this part to the extent that the interconnection of the sysems jusifies treating them as a single system for monitoring purposes. Any modified monitoring shall be conducted pursuant to a schedule specified by the State and concurred in by the Administrator of the U.S. Environmental Protection Agency.

Subpart D—Reporting, Public Notification and Record Keeping

§ 141.31 Reporting requirements.

(a) Except where a shorter reporting period is specified in this part, the supplier of water shall report to the State within 40 days following a test, measurement or analysis required to be made by this part, the results of that test, measurement or analysis.

(b) The supplier of water shall report to the State within 48 hours the failure to comply with any primary drinking water regulation (including failure to comply with monitoring requirements) set forth in this part.

(c) The supplier of water is not required to report analytical results to the State in cases where a State laboratory performs the analysis and reports the results to the State office which would normally receive such notification from the supplier.

§ 141.32 Public notification.

(a) If a community water system fails to comply with an applicable maximum contaminant level established in Subpart B, fails to comply with an applicable testing procedure established in Subpart C of this part, is granted a variance or an exemption from an applicable maximum contaminant level, fails to comply with the requirements of any schedule prescribed pursuant to a variance or exemption, or fails to perform any monitoring required pursuant to Section 1445 (a) of the Act, the supplier of water shall notify persons served by the system of the failure or grant by inclusion of a notice in the first set of water bills of the system issued after the failure or grant

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and in any event by written notice within three months. Such notice shall be repeated at least once every three months so long as the system's failure continues or the variance or exemption remains in effect. If the system issues water bills less frequently than quarterly, or does not issue water bills, the notice shall be made by or supplemented by another form of direct mail.

(b) If a community water system has failed to comply with an applicable maximum contaminant level, the supplier of water shall notify the public of such failure, in addition to the notification required by paragraph (a) of this section, as follows:

(1) By publication on not less than three consecutive days in a newspaper or newspapers of general circulation in the area served by the system. Such notice shall be completed within fourteen days after the supplier of water learns of the failure.

(2) By furnishing a copy of the notice to the radio and television stations serving the area served by the system. Such notice shall be furnished within seven days after the supplier of water learns of the failure.

(c) If the area served by a community water system is not served by a daily newspaper of general circulation, notification by newspaper required by paragraph (b) of this section shall instead be given by publication on three consecutive weeks in a weekly newspaper of general circulation serving the area. If no weekly or daily newspaper of general circulation serves the area, notice shall be given by posting the notice in post offices within the area served by the system.

(d) If a non-community water system fails to comply with an applicable maximum contaminant level established in Subpart B of this part, fails to comply with an applicable testing procedure established in Subpart C of this part, is granted a variance or an exemption from an applicable maximum contaminant level, fails to comply with the requirement of any schedule prescribed pursuant to a variance or exemption or fails to perform any monitoring required pursuant to Section 1445(a) of the Act, the supplier of water shall given notice of such failure or grant to the persons served by the system. The form and manner of such notice shall be prescribed by the State, and shall insure that the public using the system is adequately informed of the failure or grant.

(e) Notices given pursuant to this section shall be written in a manner reasonably designed to inform fully the users of the system. The notice shall be conspicuous and shall not use unduly technical language, unduly small print or other methods which would frustrate the purpose of the notice. The notice shall disclose all material facts regarding the subject including the nature of the problem and, when appropriate, a clear statement that a primary drinking water regulation has been violated and any preventive measures that should be taken by the public. Where appropriate, or where designated by the State, bilingual notice shall be given. Notices may include a balanced explanation of the significance or seriousness to the public health of the subject of the notice, a fair explanation of steps taken by the system to correct any problem and the results of any additional sampling.

(f) Notice to the public required by this section may be given by the State on behalf of the supplier of water.

(g) In any instance in which notification by mail is required by paragraph (a) of this section but notification by newspaper or to radio or television stations is not required by paragraph (b) of this section, the State may order the supplier of water to provide notification by newspaper and to radio and television stations when circumstances make more immediate or broader notice appropriate to protect the public health.

§ 141.33 Record maintenance.

Any owner or operator of a public water system subject to the provisions of this part shall retain on its premises or at a convenient location near its premises the following records:

(a) Records of bacteriological analyses made pursuant to this part shall be kept for not less than 5 years. Records of chemical analyses made pursuant to this part shall be kept for not less than 10 years. Actual laboratory reports may be kept, or data may be transferred to tabular summaries, provided that the following information is included:

(1) The date, place, and time of sampling, and the name of the person who collected the sample;

(2) Identification of the sample as to whether it was a routine distribution system sample, check sample, raw or process water sample or other special purpose sample;

(3) Date of analysis;

(4) Laboratory and person responsible for performing analysis;

(5) The analytical technique/method used; and

(6) The results of the analysis.

(b) Records of action taken by the system to correct violations of primary drinking water regulations shall be kept for a period not less than 3 years after the last action taken with respect to the particular violation involved.

(c) Copies of any written reports, summaries or communications relating to sanitary surveys of the system conducted by the system itself, by a private consultant, or by any local, State or Federal agency, shall be kept for a period not less than 10 years after completion of the sanitary survey involved.

(d) Records concerning a variance or exemption granted to the system shall be kept for a period ending not less than 5 years following the expiration of such variance or exemption.

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